WATER QUALITY INVESTIGATION PROGRAM

INVESTIGATION OF WATER QUALITY

IN

AGRICULTURAL DRAINS OF THE

CENTRAL VALLEY

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1. INTRODUCTION

Many publications and reports document that runoff from agricultural lands impact surface water quality in California (e.g. see review article of de Vlaming et al., 2000 and also Foe and Connor, 1989, 1991; Finlayson *et al*., 1991; Norberg-King *et al.*, 1991; Foe and Sherpline, 1993; Foe 1995; Kuivila and Foe, 1995; MacCoy *et al.*, 1995; Deanovic *et al.* 1996, 1998; Ross *et al.*, 1996; Domagalsky *et al.*, 1997; Kratzer, 1997; de Vlaming *et al.*, 1998; Dubrovsky *et al.*, 1998; Foe *et al.*, 1998; Werner *et al*., 2000; Larsen *et al.*, 1998a, b; Panshin *et al.*, 1998; Hunt *et al.*, 1999, 2003; Anderson *et al.*, 2002, 2003a, b; de Vlaming, 2002; Holmes and de Vlaming, 2003). In relation to these findings and to recent changes in the California Water Code, the Central Valley Regional Water Quality Control Board (CVRWQCB) is re-evaluating its regulatory program for runoff (discharges) from irrigated agricultural lands, primarily irrigation return flows (surface runoff and subsurface drainage) and storm water runoff.

Purpose: To gain a more complete understanding of the relationship between surface water quality and agricultural runoff, the State Water Resources Control Board (SWRCB)/CVRWQCB contracted the University of California, Davis Aquatic Toxicology Laboratory (UCD ATL) to conduct this investigation. The UCD ATL has 17 years experience in monitoring and assessing California agriculture-dominated waterways and is devoted to objectivity, integrity, and providing high quality, reliable data (http://www.vetmed.ucdavis.edu/apc/atl/, [About]).

The objectives of this pilot project include: (1) Evaluation of water quality, primarily through the use of aquatic species toxicity testing, in a limited number of agricultural drains in the San Joaquin River and Sacramento River watersheds, (2) Identification of the causes (e.g., sediment, contaminants, salt, etc.) of any water quality impairments, (3) Determination of the sources of contaminants based on the identified causes of impairments, and (4) Use the data and information gained in this investigation as a basis for designing and recommending approaches to future monitoring and assessment of agricultural runoff and drainage waters. Drawing conclusions regarding impacts of agricultural drainage on aquatic biota in waterways of California of the entire Central Valley is not an objective of this study.

2. SAMPLING

A. SITES

The primary criteria for site selection were: (1) Drainage dominated by agricultural irrigation return flow during March though September period, (2) Land use patterns surrounding the site predominated by mixed field crops (except for two sites where the primary land use is rice culture), and (3) Site is at a location near where the drainage water is discharged into a creek or river. Because this is a pilot project intended to examine water quality in irrigation return water, there was no intent to select sites representative and inclusive of all agricultural drainage throughout the Central Valley. Nor was there intent to select equal numbers of sites in the counties of the Central Valley. Funding level limited the total number of sites that could be investigated. Thus, the intent is to investigate fewer sites more intensely. Dispersing sites widely throughout the Central Valley would necessitate multiple field crews and considerable time in the field. Thus, to conserve funds for actual testing, sites are clustered in counties near UCD ATL. Locating sites in this manner also facilitates testing water samples sooner after collection.

Tables 1 and 2 (Appendix A) list the sampling sites in the San Joaquin River and Sacramento River watersheds, respectively. Maps of the individual sites are included in Appendix B. Samples will be collected from 11 sites within the Delta and San Joaquin River watershed and 13 sites in the Sacramento River watershed.

B. SCHEDULE

The focus of this pilot study will be on water quality of irrigation return water in agricultural drains. Sampling will be restricted to the 7 month irrigation season (March-September 2003). The limited time-frame and funding for this project constrains the number of sites that can be sampled and the frequency of sampling (and testing). Infrequent sampling of a large number of sites cannot adequately characterize water quality in agricultural drain water. Thus, the objective in this pilot project is to focus on a relatively small number of sites with more frequent sampling and testing. The project will begin with a fixed sampling schedule, each site being sampled every three weeks (beginning in March). If toxicity is observed with either test species in a site sample, that site will be re-sampled within 48 hours. The increased frequency of sampling will

continue until no toxicity is observed in samples from that site. The intent of this sampling strategy is to obtain an estimate of the duration of toxicity. The significance and ecological relevance of toxicity at a site are related to duration, magnitude, and frequency of that toxicity. This sampling strategy will assist in addressing this issue.

The preference is to focus sampling efforts on periods of peak irrigation, especially the first major irrigation of the season. Sampling during these periods may provide the most informative data. To coordinate sampling with peak irrigation events, UCD ATL will need the cooperation of irrigation districts in the areas where sampling sites are located.

C. PROCEDURES

Collection- Samples will be collected as sub-surface grabs from mid-channel (whenever possible) in pre-cleaned, 1-gallon, amber glass bottles. Each container will be rinsed three times with site water prior to final filling. The amber glass bottles appear to preserve sample integrity better than plastic containers. Amber glass also minimizes photo-degradation of the sample. All sample containers are pre-cleaned by the UCD ATL, following the UCD ATL Standard Operating Procedures Manual (SOP), SOP 10-1. Flow will be measured at each site.
Sample containers are labeled with site identification, collection date, and time. The sampling team will record relevant information in the field log book and in the chain of custody (COC) form including: (1) sample identification (a unique number for each sample), (2) sample location, (3) date and time of sample collection, (4) sampler's name, and (5) field instrument readings [including water temperature, pH, dissolved oxygen (DO), and electrical conductivity (EC)].
Water renewals in a toxicity test on a site sample will be from the initial grab sample. Using a single grab sample for toxicity test renewals facilitates determination of the cause(s) of toxicity (see Toxicity Identification Evaluation section). Furthermore, a single grab sample represents an ecologically relevant exposure regime for testing planktonic organisms (e.g., zooplankton and larval fish) that are transported in a water mass. The indicator species to be used in this investigation are both planktonic.

Storage - Immediately after collection, samples will be placed in an ice chest on wet ice for transport to the UCD ATL where they are stored in the dark at 4±2ºC. Toxicity tests will be initiated within 48 hours of collection. More detailed information on sampling procedure is

presented in the UCD ATL SOP Manual, SOP 5-1 and 5-2. The chain of custody form that is used to document sample collection and receiving is appended (Appendix C). These forms are maintained at UCD ATL for seven years.

3. TOXICITY TESTING

A. BACKGROUND AND GENERAL PROCEDURES

Background- Aquatic species toxicity testing will be used as the primary water quality assessment procedure in this investigation. All monitoring and assessment procedures have strengths and limitations. Several strengths and limitations of aquatic species toxicity testing have been summarized by de Vlaming and Norberg-King (1999) and de Vlaming et al. (2000). Single species toxicity tests are an integrative and direct measure of aggregate toxicity of multiple chemicals; they measure bioavailability of toxic substances; they afford reliable, repeatable, and comparable results; they are highly standardized with specific quality assurance requirements; they can be performed relatively quickly and inexpensively; and in combination with Toxicity Identification Evaluations they can identify the cause(s) of toxicity. In a majority of cases ambient water toxicity test results have provided a reliable qualitative prediction of impacts to instream biota (e.g., Waller et al., 1996; de Vlaming and Norberg-King, 1999; de Vlaming et al., 2000, 2001).

General procedures- Toxicity testing will follow the 4-day static renewal procedures described in Methods for Measuring Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms (US EPA, 2002). In this study all samples will be tested in the *Ceriodaphnia dubia* (a cladoceran, zooplankton species) and larval *Pimephales promelas* (a cyprinid minnow) tests. Aspects of these procedures that differ from the US EPA methods, and the rationale for using them, are outlined below.

While US EPA methods do not specifically recommend aeration of the renewal water, the UCD ATL protocols include aeration. This deviation is employed because the ambient samples tested at the UCD ATL frequently require aeration to prevent oxygen super-saturation. Aeration time will be limited until sample comes to 102% saturation to minimize the loss of potential toxicity due to volatile toxicants.

Single species tests- The UCD ATL uses control waters made per UCD ATL SOP 7-1 through 7-4. Sierra Springs™ water amended to EPA moderately hard (SSEPAMH) is used as the control water for the *Ceriodaphnia dubia* test. Deionized water amended to EPA moderately hard (DIEPAMH) is used as the control for the minnow test. Samples from agriculture-dominated waters are sometimes characterized by relatively high salinity (as determined by electrical conductivity (EC) measurements. A second control will be included when EC exceeds 2000 µmhos. This second control will match the highest EC in a series of samples. In many years of testing water samples from the San Joaquin River and Sacramento River watersheds UCD ATL has not observed that salinity was the cause or a contributor to test species mortality.

Ceriodaphnia dubia: Cultures originally obtained from Aquatic Research Organisms, New Hampshire, are maintained at the UCD ATL (SOP 2-3 and 3-1). Test organisms employed are less than 24 hours old and are derived asexually from one animal.

The *Ceriodaphnia dubia* acute tests consist of four replicate glass vials containing 18 ml of sample with five organisms each. Ideally, the US EPA 7-day test procedure should be applied to water quality evaluation. Limited funding precludes use of the 7-day test. Therefore, acute toxicity tests will be conducted. Use of the 96-hour acute test (US EPA, 2002) was selected to increase sensitivity and because aquatic biota can be exposed to contaminants for 96 or more hours. The 96-hour test decreases the probability of false positives and underestimating toxicity. Tests are initiated with less than 24-hour-old *Ceriodaphnia,* born within a 20-hour period. *Ceriodaphnia* are fed a mixture of *Selenastrum* and YCT (a mixture of yeast, CEROPHYLL®, and trout chow) before test initiation and four hours prior to test renewal. US EPA suggests usage of plastic cups and water renewal at 48-hours with a 2-hour feeding. The UCD ATL opts to use glass vials rather than plastic. Daily renewal of test water is employed at the UCD ATL to minimize contaminant degradation. No food is added to the daily renewal waters to minimize toxicant sorption to food particles. *Ceriodaphnia* are transferred into a new vial of fresh test solution daily. Tests are conducted at 25 ± 2°C with a 16-hour light: 8-hour dark photoperiod. Mortality is assessed daily and at test termination. Test parameters are summarized in Table 1 (Appendix D).

Pimephales promelas: Larvae, hatched in transport, are obtained from AquaTox, Inc. Arkansas (SOP #2-4). When the larvae arrive, they are acclimated in a container with DIEPAMH water which is then placed into a

25°C bath and slow, constant aeration is applied. Testing is initiated after acclimation and before the larvae are more than 48 hours old.

The larval *Pimephales promelas* 96-hour tests consist of four replicate 600 ml beakers, each containing 250 ml of sample and 10 minnows. Less than 48-hour-old minnows, born within a 24-hour period are employed. Minnows are fed before test initiation and twice daily while on test with brine shrimp *Artemia* nauplii. US EPA suggests water renewal at 48-hours and a single feeding at 48-hours. Due to the potential for rapid contaminant degradation, sample waters are renewed daily to ensure a more consistent toxicant concentration. UCD ATL feeds half the US EPA suggested amount twice daily to reduce bacterial growth in test chambers. Approximately 80 % of the test water is renewed daily. Test water is incubated in a water bath at 25 ± 2°C under ambient laboratory light with a 16-hour light: 8-hour dark photoperiod for four days. Mortality is measured daily at the time of water renewal and at test termination. Test parameters are summarized in Table 2 (Appendix D).

B. DATA MANAGEMENT

Reduction and Storage - All raw toxicity test, TIE, and sample water quality data will be recorded in non-erasable ink on standardized printed data sheets. The raw data are entered into spreadsheets and manipulated with statistical programs, then photocopied and used when performing data interpretations. All data will be submitted to the Regional Board contract manager as part of the corresponding project reports. Summary tables will be generated for the toxicity tests, TIEs, and the water quality parameters. All tables and statistical analyses will be proofread and checked for quality assurance. All data will be filed and stored on site in a secure cabinet for seven years.

Statistical Analysis - Each sample will be characterized by descriptive statistics indicating the mean response and variation among replicates. Statistical comparisons will consist of t-tests that compare the response of test organisms in sample water to the response in laboratory dilution water controls.

Toxicity is defined as a statistically significant mortality difference ($p<0.05$) in an ambient sample compared to laboratory control(s). Specifically, acute toxicity in the *Ceriodaphnia* and larval *Pimephales* assays is defined as statistically significant mortality within 96 hours in a test sample compared to the laboratory control.

Ceriodaphnia and larval *Pimephales* mortality data will be transformed with the arsine of the square root transformation and analyzed with Bartlett's Test for homogeneity of variance using Microsoft Excel. If data are characterized by homogeneous variance, the transformed data will be analyzed using an Analysis of Variance and Dunnett's mean separation tests using SAS Institute Statview. If the data consists of heterogeneous variance, the raw mortality data will be transformed to relative ranks and then analyzed using an Analysis of Variance and Dunnett's mean separation test using SAS Institute Statview.

These statistical analyses differ from those outlined in US EPA (2002). US EPA statistical procedures were designed for whole effluent toxicity testing in which all samples are tested in a dilution series. The approach to be taken during this study will be to assess water quality at particular sites compared to laboratory control water. Because these tests will not include a dilution series, the US EPA statistical protocols are not appropriate for the data obtained during this study.

C. QUALITY ASSURANCE

Quality assurance measures will be included in this project to ascertain the reliability of data gathered including whether the UCD ATL testing can be duplicated and to assess whether test species are responding typically, relative to historical test results at the UCD ATL. To assess repeatability (precision), laboratory control duplicates, field duplicates, and toxicant-spiked duplicates will be tested. To determine whether test species are responding typically during this study, toxicant-spiked samples will be tested and reference toxicant tests will be conducted. The various components of QA activities are summarized below.

Positive control tests- At least one positive control (i.e., reference toxicant) test will be performed monthly. Reference toxicant tests determine test species sensitivity to a toxicant and whether the test species is reacting typically (within a predetermined range) to that toxicant. These tests will include a laboratory control and a toxicant dilution series in laboratory control water. The LC_{50}/EC_{25} for each reference toxicant test is compared to the UCD ATL running mean to ascertain whether it falls within the acceptable range. The US EPA acceptable range is plus or minus two standard deviations around a running mean. For this project, if a reference toxicant test result does not fall within this acceptable range, results of associated toxicity tests will be considered suspect and identified in interim and final reports.

Duplicate QA samples- Treatments that investigate precision will include ambient water blind duplicates for assessing laboratory performance, toxicant spikes into laboratory control water to assess organism sensitivity, matrix (i.e., ambient water from the Sacramento and San Joaquin River watersheds) spikes for assessing matrix effects on a known toxicant, and laboratory control water trip blanks to assure that transport does not cause toxicity. Ten percent of all samples will be randomly selected for these quality assurance procedures. Ambient water duplicates will be collected using the same procedures as the initial/primary sample, but will be labeled with a different identification so that laboratory technicians can not recognize duplicates. Test organisms are expected to perform similarly between the sample and it's duplicate. The matrix spike and matrix spike duplicate are prepared in the laboratory from a randomly chosen site sample. The laboratory spike is laboratory control water amended with the same toxicant as the matrix spike and matrix spike duplicate. In this project, duplicates will be compared by statistical analysis to assess differences. If statistical differences ($p<0.05$) are observed between duplicates the original data will be considered suspect. Results of these analyses will be presented in interim and final reports.

Test acceptability criteria- Test acceptability for all *Ceriodaphnia* and larval *Pimephales* 96-hour tests requires 90% or greater survival in the controls. When the control performance does not meet test acceptability criteria, all data from the test are rejected. The percentage of 7-day tests in which test species control performance met test acceptability criteria at the UCD ATL was evaluated using data from 40 randomly selected tests (per test species) conducted from January 1999 through January 2001. Meeting test acceptability rates were (n=40): 97.5% for *Ceriodaphnia dubia* tests and 92.5% for larval *Pimephales promelas* tests. For *Ceriodaphnia* 96-hours tests, 100% of the tests met the acceptability criteria (n=24).

Deviations and corrective actions- Tests are conducted according to test conditions recommended by the US EPA (2002) with the exception of those reported herein. Beyond those identified herein, deviations from these recommended conditions are reported to the UCD ATL QA Officer. The laboratory director and contract manager will be notified, within 72 hours of these deviations.

Failure to meet QA criteria can have several outcomes. In some cases, corrective action can occur and in other cases it cannot. For example, if test acceptability criteria are not met with a sample, corrective action will be a re-test of the sample or substitution of a sample collected at

the same site at a later date. If samples arrive at the UCD ATL at >10°C or if testing cannot be initiated within the 48 hour maximum sample holding time, the fate of those samples will be determined by the laboratory director on a case by case basis. In the event of standard operating procedure (SOP) deviations, a deviation form will be prepared and the Contractor notified. UCD ATL SOP references are summarized in Table 3 (Appendix D).

Best professional judgment will be used in interpretation of results obtained when deviations in the test conditions have occurred. All deviations and associated interpretations will be reported in interim and final reports.

Precision- Precision is the degree to which independent analyses of a given sample agree with one another; it is the reproducibility, consistency, and repeatability of results. Though precision criteria have not been developed for these toxicity tests, the UCD ATL assesses precision through several practices that include matrix spike duplicates, field duplicates, and inter-laboratory split samples. A field duplicate is a second sample collected in a separate container, immediately after the initial/primary test sample. A matrix spike refers to a field sample representative of the surface water system that has been spiked with a toxicant to achieve a predetermined concentration. A matrix spike duplicate refers to a matrix spike that is split into two sub-samples that are subsequently subjected to independent toxicity testing.

The relative percent difference (100x{ |Duplicate 1 - Duplicate 2| / [(Duplicate 1 + Duplicate 2)/2]}) between field duplicates at the UCD ATL has been calculated for several toxicity testing and water quality parameters (Table 1).

Toxicity testing endpoints for field duplicates also have been evaluated to determine the frequency that the UCD ATL data show equivalent results. Paired duplicates were statistically compared to determine equivalent results. Results can agree (both non-toxic or both toxic) or disagree (one toxic and the other non-toxic). Table 2 illustrates the frequency that field duplicates in chronic toxicity tests were in agreement (data collected between July 1999 and November 2002). These data demonstrate that there is a high degree of toxicity testing precision at the UCD ATL. Over the last eight years, toxicity test false positives at the UCD ATL have been very infrequent as demonstrated by re-test, TIEs, and chemical analyses. In samples identified as toxic in initial tests, less than two percent were possibly false positives.

Table 1. Summary of laboratory precision at the UCD ATL (July 1999-November 2002).

Test Parameter	Sample Size (n)	Average % Difference	Standard Error
Hardness	28	10.6	2.6
Alkalinity	28	8.2	2.3
pH	29	1.6	0.4
EC	29	6.6	1.7
Ammonia	27	19.0	10.3
Chronic larval *Pimephales* Mortality	22	16.1	10.71
Chronic *Ceriodaphnia* Mortality	25	2.7	3.6

Table 2. Frequency of field duplicates sharing equivalent results.

Test Parameter	Sample Size (n)	Duplicates in Agreement (%)
Ceriodaphnia Mortality (7-day test)	23	95.7
Ceriodaphnia Mortality (96-hour test)	5	100.0
Larval *Pimephales* Mortality (7-day test)	20	100.0

Accuracy- Accuracy of toxicity tests cannot be directly measured, but inferences can be made from reference toxicant tests. Historical reference toxicant testing suggests that UCD ATL toxicity testing results are accurate. See section on positive control for dealing with accuracy outliers.

D. WATER QUALITY

Various water quality parameters other than contaminants can affect toxicity test results. Thus, UCD ATL monitors several factors that could confound test results to aid in toxicity data interpretation. Water quality parameters of temperature, electrical conductivity (EC), hardness, alkalinity, pH, and dissolved oxygen (DO) are measured on all samples at test initiation; temperature, pH and DO are measured at the 24-hour sample renewals. Laboratory pH is measured with a Beckman IS 425 pH meter, DO is measured with a YSI model 58 oxygen meter with a 5700 series probe, and EC is measured with a YSI model 33 EC meter. All meters are calibrated daily according to the manufacturers' instructions. Ammonium is measured on all samples within 24 hours of receipt with an Aquaquant® ammonium kit (EM Science). Unionized ammonia is calculated using the formula in US EPA Update of Ambient Water Quality Criteria for Ammonia (1999). Turbidity and suspended sediment concentration (SSC) will be measured on all samples within 10 days of receipt. Hardness and alkalinity are measured utilizing titrimetric methods. Turbidity is measured with a HACH® spectrophotometer model 2100A Turbidimeter. Instrument calibration and preventative maintenance are summarized in Appendix E.

SSC is used to determine the suspended material in water samples. The UCD ATL will follow the methods outlined in ASTM D3977-97 (1999). One liter of sample water is passed through a 1.5 micron glass fiber filter so that all of the sediment is retained. The filter and its contents are dried and weighed in order to calculate the SSC. Total Organic Carbon (TOC) and Dissolved Organic Carbon (DOC) will be determined in the laboratory of Dr. Tom Young (Civil and Environmental Engineering) using a Shimadzu model TOC-5050 with an ASI-5000 autosampler/autoinjector based on EPA Procedure 415.1.

E. CONSIDERATIONS AND CONSTRAINTS

US EPA recommends that toxicity tests be initiated within 36 hours of sample collection. The UCD ATL makes every effort possible to initiate tests within 36 hours of sample collection. Although storage at 4 ± 2°C in darkness generally slows or inhibits degradation of toxicants, increased holding times can result in reduced concentration(s) of some sample contaminants. Degradation and/or adsorption of toxicants on container surfaces during the holding period also can result in underestimation of toxicity and yield false negatives. Sampling will be timed to minimize holding time. Results of tests where samples were held 48 hours or more prior to test initiation will be specifically identified in data reports and in the final report.

F. REPRESENTATIVENESS

Representativeness refers to the degree to which data accurately represent responses of resident populations at the site where the sample was collected. Most UCD ATL projects are intended to measure toxicity and estimate adverse impacts to resident aquatic ecosystem biota.

The US EPA Technical Support Document (1991a) summarizes several studies that support the use of EPA's three freshwater chronic toxicity protocols. These species are generally considered appropriate surrogates (indicator species) for indigenous freshwater biota. Toxicity test results will be considered representative of toxicity at the sampling site if the sampling protocol is followed, tests are initiated within the holding time and laboratory water chemistry results are within ranges observed in the field. Recent review articles conclude that US EPA toxicity test results are effective predictors of impacts to resident biota (Waller *et al.*, 1996; de Vlaming and Norberg-King, 1999). Thus, the UCD ATL considers toxicity test results to be indicative of resident species responses when appropriate evaluation of field exposure is included.

Estimating risk to indigenous aquatic biota using ambient sample toxicity involves estimation of magnitude, duration of exposure, and the geographic extent of the toxicity. The limited time-frame and budget for this investigation constrains the quantity of these types of data that can be amassed. Furthermore, while the sampling sites for this pilot project were selected to be representative of particular agriculture drains, the limited number of sites is not likely to represent all agricultural drains in the Central Valley or California. Thus, the intent of this project is not to draw conclusions regarding impacts of agricultural drainage on aquatic biota in California or the entire Central Valley. That is, the information and data gathered in this investigation will be a component in UC's recommended design and approaches for operating future monitoring of runoff from agricultural lands.

G. COMPARABILITY

Comparability relates to similarity of data from different data sets and sources; it is an indication of the confidence with which one data set can be compared to another. With the exception noted herein, the UCD ATL strictly documents and adheres to US EPA test protocols, UCD ATL SOP's, QA measures outlined herein, and acceptable reference toxicant test results. Therefore, the laboratory results obtained in one project can be compared to results from previous UCD ATL projects as well as from other laboratories using the US EPA procedures.

H. TEST SENSITIVITY

Test sensitivity refers to the ability to distinguish a statistical difference between test organism response in laboratory control water compared to an environmental sample. Test sensitivity is frequently expressed as the percent difference between the control and environmental sample that can be detected. The level of effect that can be detected will vary, depending on control performance, variability among replicates, the test species, and endpoint measured. In the tests to be used in this investigation UCD ATL typically has been able to detect approximately 20% or more difference from controls. At this time, UCD ATL does not have acceptability criteria for test sensitivity. The lower the test sensitivity, the greater the probability of false negatives (sample is toxic but test does not detect). Test sensitivity can be increased by increasing the number of replicates. That, in turn increases the costs of testing. UCD ATL will identify test results in which the ability to distinguish a difference between control and ambient water sample was 25% or greater.

I. DATA AUDITS

All data reported for this project will be subject to a 100% check for errors in transcription, calculation, and computer input by the UCD ATL QA Officer. Additionally, the QA Officer will review all sample logs and data forms to ensure that requirements for sample holding times, sample preservation, sample integrity, data quality assessments, and equipment calibration have been met. At the discretion of the laboratory director, data that do not meet these requirements will either not be reported or will be reported with an explanation of associated problems.

J. PERFORMANCE AND SYSTEM AUDIT

The Central Valley Regional Board or their designee may conduct inspections of the physical facilities, operational systems, and operating procedures at the UCD ATL. The inspections can be conducted while toxicity tests are being performed; the facility should be given 24-hour notice of the inspections.

4. TOXICITY IDENTIFICATION EVALUATIONS

Toxicity testing data are of limited value if the cause(s) (defined as a component of the water sample) of that toxicity is/are unknown. That is, mitigation activities, be they volunteer or regulatory based, are greatly facilitated when the cause(s) of toxicity is/are known. Thus, a major effort in this study will be to specifically identify the cause(s) of toxicity in toxic samples. Toxicity identification evaluations (TIEs) consist of physical, chemical, and toxicological manipulations designed to identify the specific toxicant or class of chemicals responsible for toxicity observed in a sample (US EPA, 1991b). TIEs will be performed on either test species that exhibits 50% or greater mortality in the initial test. TIEs will be performed to the extent (Phase II or Phase III) necessary to determine the cause(s) of toxicity.

A. DILUTION SERIES

Dilution series tests will be performed to determine the magnitude/potency of toxicity in a toxic sample. Results of these tests will be used to estimate the toxic units (TUs) in a toxic sample. Toxic units are estimated by dividing the 100% sample by the lowest sample dilution causing toxicity. For example, if the sample diluted to 25% causes toxicity, the sample consists of at least four TUs of toxic substances. TUs contributed by individual toxic chemicals can also be estimated. In this context, a TU is defined as the concentration of a specific chemical present in a sample divided by the 96-hour LC_{50} concentration for the species of interest. An LC_{50} is defined as the concentration of a chemical that causes 50% mortality in 96 hours. Toxic units can be added when multiple toxicants are present (assuming that the individual toxic compounds act additively) to equal the total number of toxic units. Toxic units contributed by individual toxicants can be compared to toxic units determined by dilution of the ambient water sample. Dilution series tests will be performed on samples causing 100% mortality within 48-hours to either *Ceriodaphnia* or larval *Pimephales*. Dilutions will consist of 100, 50, 25, 12.5, and 0% of the sample. Dilutions are made with control water for each respective species.

B. PHASE I TIES

The purpose of Phase I TIEs is to identify the class(es) of contaminant(s) causing the toxicity. The toxicity tests associated with TIE procedures are performed as described above; additional sample manipulations are performed to reveal the cause(s) of toxicity. Solid phase extraction (SPE) columns remove nonpolar organic chemicals from aqueous test samples as it is passed through. Toxic samples are passed through an SPE column and these waters are tested along with the unmanipulated sample. Control water also is passed through a SPE column and serves as one of the procedure controls. The adsorbate is then eluted with methanol and the eluate is added to control water and tested along with the appropriate method blanks. A methanol control is included in the procedures. If the toxicant is a nonpolar organic chemical, the ambient sample and control water amended with eluate will exhibit high mortality while the sample passed through the SPE column results in reduced or no mortality. In some cases, binding of metals to organic and inorganic ligands in samples will reduce the bioavailability of metals. The extent of metals binding to organics can be estimated by comparing the toxicity of the sample before and after solvent extraction, since solvent extraction removes organic-bound metals. Disodium Ethylenediamine Tetraacetate (EDTA) and Sodium Thiosulfate (STS) bind to various metals, making them unavailable to biota. Three concentrations of each EDTA and STS will be added separately to toxic samples and tested along with the appropriate controls. If the toxicant is one of these metals, the ambient sample will exhibit high mortality while the ambient sample amended with EDTA or STS results in reduced or no mortality. Air stripping sometimes reduces or removes surfactants and/or ammonia from waters. Toxic samples will be air stripped and tested along with the appropriate control. If the toxicant is a surfactant, the ambient sample will exhibit high mortality while the air stripped sample usually results in reduced or no mortality. Additionally, in the *Ceriodaphnia* Phase I TIE, samples are amended with piperonyl butoxide (PBO). PBO inhibits or reduces toxicity caused by metabolically activated organophosphorous (OP) insecticides such as diazinon, chlorpyrifos and malathion (Bailey *et al.*, 1996). 100 μg/L PBO is added to the toxic samples. The 'original' ambient test sample and the ambient test sample amended with PBO are tested along with the appropriate controls in a toxicity test. If the toxicant is a metabolically activated OP insecticide, the ambient test sample will exhibit high *Ceriodaphnia* mortality while the ambient test sample amended with PBO results in reduced or no *Ceriodaphnia* mortality.

C. PHASE II TIES

The purpose of Phase II TIEs is to identify the constituent(s) causing or contributing to the toxicity. If the Phase I TIE suggests that the toxicity is due to cationic metals (e.g. removal of toxicity by EDTA and STS), the sample will be analyzed for metals according to standard US EPA analytical procedures. The metals detected will be spiked into laboratory water (with water quality characteristics adjusted to match the test sample) at the concentrations measured in the test samples. If metals are implicated, the toxicity of the test sample and the metal-spiked laboratory water sample should closely match. If the Phase I TIE suggests toxicity due to nonpolar organic constituents, the sample will be concentrated on SPE columns and fractionated by high-performance liquid chromatography (HPLC). HPLC fractions that exhibit toxicity will be subjected to analysis using advanced instrumentation (see Section E) to identify the suspect toxicant(s).

D. PHASE III TIES

The purpose of Phase III procedures is to confirm the contaminant cause (s) of toxicity. Mortality rates are compared between paired dilution series consisting of the ambient sample and suspected-toxicant amended control water. The latter is amended with the toxic constituent(s) to match the concentration(s) measured in the ambient sample. Sample concentrations within the paired dilution series are selected to include the anticipated NOEC and LOEC, and vary depending on the number of toxic units in a sample. The cause(s) of toxicity is/are confirmed when the mortality is equivalent in matching samples of the two dilution series (i.e., dose-response curves are the same).

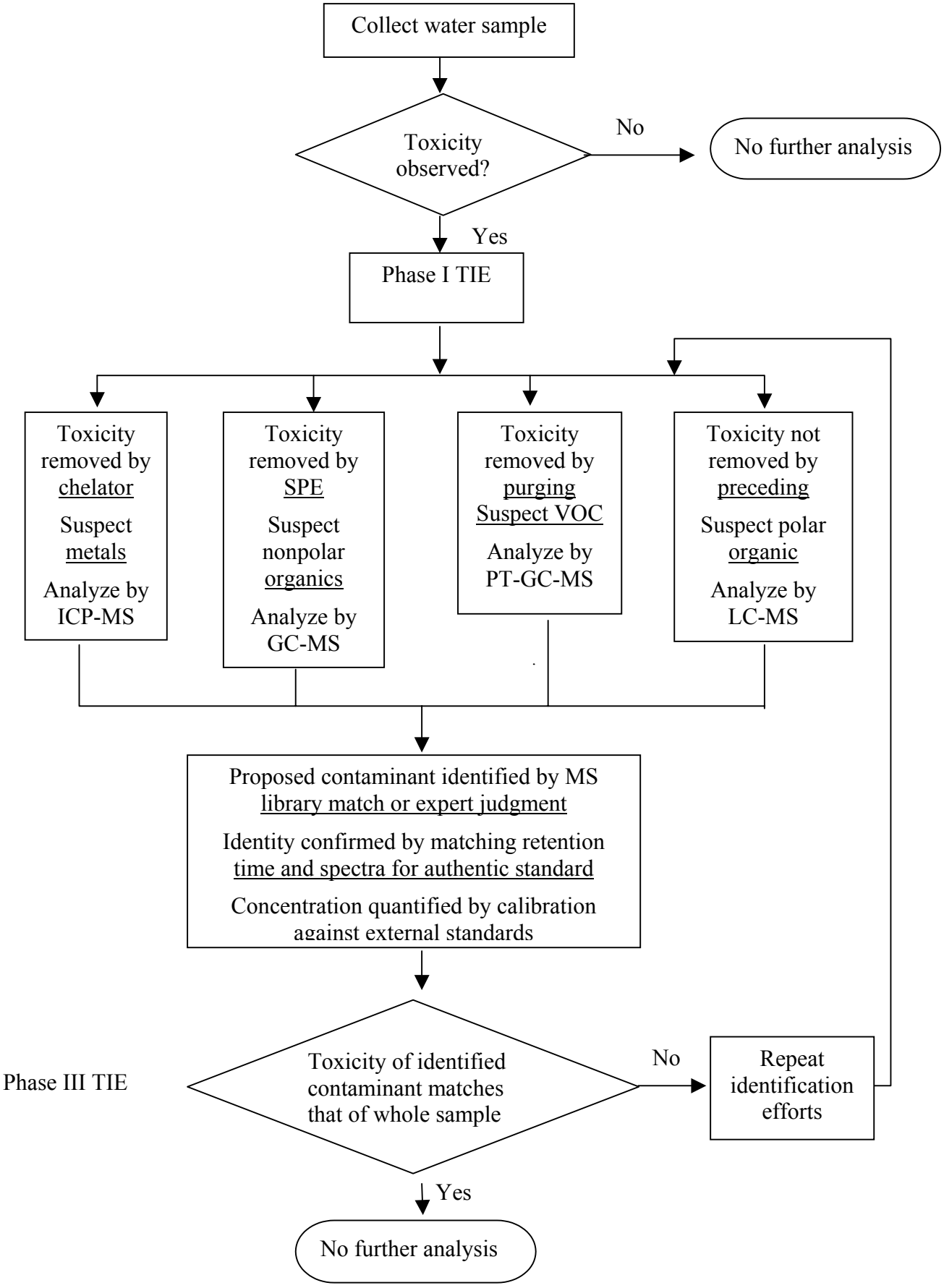
E. CHEMICAL ANALYSES

Laboratory qualification- The goal of all trace chemical analyses conducted for this project will be to identify the cause(s) of any toxicity identified. The chemical analyses are therefore integral to the toxicity testing, and the primary way their accuracy will be assessed is by comparison to the results of subsequent toxicity testing. Analyses will be performed in the laboratory of Dr. Thomas Young in the Department of Civil & Environmental Engineering, University of California, Davis. Dr. Young and Dr. Peter Green, an Assistant Research Engineer in the same department, will be the lead investigators on the trace chemical analysis portion of this project, and they have many year's experience developing methods and identifying unknown compounds (e.g., Grosjean et al.,

1999; Schultz et al., 1999; Young et al., 2002). The laboratory is an advanced instrumentation facility for the analysis of environmental samples for organic compounds, metals and semi-metals of toxic concern. Available instrumentation includes two gas chromatographs (Agilent 6890) coupled to mass spectrometers, one with chemical ionization capability, a liquid chromatograph (Agilent 1000-series) coupled to a mass spectrometer via either atmospheric pressure or electrospray interfaces, and an inductively coupled plasma-mass spectrometer (Agilent 7500i) for trace element measurements. All of these instruments are equipped with autosamplers and are rigorously maintained. Additional details about all of the advanced instrumentation available within the Department of Civil & Environmental Engineering can be accessed via the internet (http://cee.engr.ucdavis.edu/faculty/young/laboratory.htm). An overview of the approach to be taken for identifying, quantifying and verifying toxicants is shown in Figure 1 and details of the approach are provided in the remainder of this section.

Identifying unknown toxicant- Mass spectrometry will be the primary means of identifying unknown toxicants; the exact approach to be used will depend on the results of the Phase I TIE described above. If a metal is the suspected toxicant because toxicity was removed by adding a chelating agent, the original sample will be analyzed by inductively coupled plasma mass spectrometry (ICP-MS). If a nonpolar organic chemical is the suspected toxic agent because toxicity was removed after passing the sample through an SPE cartridge, a solvent wash of the SPE will be analyzed by gas chromatography-mass spectrometry (GC-MS). This approach will also be followed if the Phase I TIE indicates that the suspected toxicant is a metabolically activated pesticide. If a volatile organic compound (VOC) appears to be responsible because the toxicity was removed by purging, the sample will be analyzed by purge and trap gas chromatography-mass spectrometry (PT-GC-MS). If toxicity is not removed by chelation, SPE, or purging the cause will be presumed to be a polar organic compound and analyses will be conducted using liquid chromatography-mass spectrometry (LC-MS). In each case the analyses will follow standard laboratory protocols for scanning unknown mixtures (standard operating procedures ICPMS-1, GCMS-4, PTGCMS-2, LCMS-1). Briefly, these methods rely upon matching the mass spectra obtained against a spectral database (National Institute of Standards and Technology; NIST) in the case of GC-MS or expert examination of isotope patterns in the case of ICP-MS or mass spectral motifs in the case of LC-MS to identify the suspected toxicant.

Figure 1. Flowchart describing chemical analysis approach



Laboratory standards- Authentic standards of the suspected toxicant will be from the laboratory collection of metal and organic standards, purchased in pure form from commercial sources, or isolated from commercial mixtures. This last step is expected to be required only if the suspected toxicant is an adjuvant to a commercial pesticide application. All standards from the laboratory collection are kept properly stored and will be freshly diluted to working concentrations as needed. Standards will be used to prepare a five-point calibration curve bracketing the instrument response in the initial identification. The unknown sample will be re-analyzed along with the calibration standards to provide confirmation and quantification. Confirmation of the identity of the compound will be based primarily on the mass spectral match between the authentic standard and the unknown. In the case of the chromatographic methods (GC-MS, PT-GC-MS, LC-MS) confirmation will also require a retention time match between the standard and the unknown.

Data quality objectives- The data quality objectives for the chemical analysis portion of the study are to correctly identify the toxicant (avoid false positives) and achieve accurate quantification of concentrations. Satisfaction of the first two objectives will be assessed by the results of the Phase III TIE. If the paired dose-response curves for the suspected toxicant and dilutions of the original sample match, then the toxicant's identity and quantification will be considered confirmed. If the Phase III TIE indicates that the toxicant identified in the chemical analysis does not account for all of the observed toxicity in the original sample, additional causative agents will be sought by reviewing Phase I TIE results and performing any additional analyses that might be suggested by this review. If the toxicant identified produces more toxicity than observed in the original sample, the presence of an antagonist or an error in chemical quantification will be suspected. Quantification and identification will be independently confirmed using the approaches described above. Antagonists will be sought in the form of interacting chemicals or other agents (e.g., colloidal particles, dissolved organic carbon) that may bind with or otherwise reduce the toxic effects of the suspected toxicant.

Quality control samples- For Quality Control, every third batch of samples received for chemical analysis will include a matrix spike and a matrix spike duplicate. Spikes will be chosen as environmentally relevant toxicants that might be present within the samples being analyzed. Once a suspected toxicant is identified, the holding time for that sample will be determined and compared to those required for related EPA methods. Consistent analytical procedures will be ensured by close adherence to established laboratory standard operating procedures. Dr. Peter

Green will perform or supervise all laboratory work. He is responsible for Quality Assurance of chemical analyses. For the computer-controlled analytical instruments ICP-MS, LC-MS and GC-MS, self-check or validation software is resident with the instrument, and will be used periodically to track instrument performance. ICP-MS is tuned every 24 hours. LC-MS and GC-MS are tuned once each week. In all cases, procedures follow manufacturer specifications. GC-MS, LC-MS, and ICP-MS analysis data will be reduced using the corresponding Agilent Chemstation software. All data are tentative until completely reviewed for quality assurance as described above. After analysis, the remaining volume of sample will be re-sealed and re-stored for repeat and/or follow-up analysis as needed. When instrument tuning or calibration cannot be confirmed, all associated data will be discarded.

Data reduction and storage- The methods described above will generate a complex array of information that is necessary to understand these potentially complex samples. With the wide range of information likely to be obtained, unexpected patterns might surface. The possibility of unforeseen results does not compromise the project. Such data will be harnessed to better understand the samples, thereby improving the overall success of the project. In short, because the study design is not limited to pre-chosen analytes, unexpected results (i.e., unsuspected toxicants) have a higher likelihood of being detected. The expectation is to meet or exceed requirements of comparable EPA methods (e.g., Miller et al. - http://www.sacriver.org/subcommittees/toxics/documents/AlgaeTIEReport.pdf).

Standard laboratory data-logging practices such as page-numbered notebooks and entries in ink will be followed. However, the majority of information from this project will consist of digital data acquired by instruments, or the result of computation using the data, and will not be present in traditional laboratory notebooks. For digital data, the laboratory will use a system of data backup in which either or both of the following will be performed: (a) data is copied to another computer (via FTP, data cartridges, E-mail attachments, or other means); (b) data backup onto CD-ROMs or other appropriate backup media. All data from the project (digital or paper copies) will be maintained for a minimum of seven years following completion of the project.

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APPENDIX A

Sample Sites

Table 1. Sample locations in the San Joaquin River/Delta watersheds

Site Name (County)	GPS Coordinates (Lat/Long,WGS84)	Directions	Parking	Sampling Location	Cropping
Beaver Slough @ Blossom Rd (San Joaquin Co.) Site 1	38.20421°N 121.44706°W	I-5 South of Sacramento Exit I-5 @ Walnut Grove Rd. Head West (Left) on Blossom Rd	Turnout @ SW corner (do not park in front of private drive)	West side of bridge	alfalfa, asparagus, mixed row, grapes
Next site	Continue south on Blossom Rd to return to I-5. Potential site: unnamed slough where Blossom Rd makes a 90° turn to Peltier Road. Sample if ag return water flows.				
Unnamed Slough @ Woodsbro Rd and Hwy 4 (San Joaquin Co.) Site 2	37.92680°N 121.36697°W	I-5 to west on Hwy 4	South side of Hwy 4	South side of Hwy 4 off of pipe	alfalfa, asparagus, mixed row
Next site	Head west on Hwy 4 & turn right on to Whiskey Slough Rd				
Return irrigation drain @ McDonald & Boultin Rds (San Joaquin Co.) Site 3	37.96983°N 121.46227°W	Hwy 4 to Whiskey Slough Rd. Right on Holt Rd. Left on McDonald Rd to Boultin Rd	West side of McDonald Rd on Boultin Rd	Downstream McDonald Rd culvert	corn, asparagus, mixed row
Next site	Continue on McDonald Rd, right bend onto Nueger Bauer Rd.				
SJR source water into canal @ Holt Rd & Nueger Bauer Rd (San Joaquin Co.) Site 4	37.99402°N 121.42045°W	Follow Nueger Bauer along river to Holt Rd	East side of Holt, south of T	Off of pump platform	mixed ag sources
Next site	South on Holt Rd, east (left) on McDonald Rd, south (right) onto Inland Dr.				

Site Name (County)	GPS Coordinates (Lat/Long,WGS84)	Directions	Parking	Sampling Location	Cropping
Drain @ 11751 Wing Levee Rd (San Joaquin Co.) Site 5	37.85659°N 121.37801°W	South on Inland Dr from Hwy 4. Follow Inland left to a right on Stark Rd. Right on Howard Rd over bridge to a quick left on Wing Levee	60 meters south of mailbox	Off of pump landing	mixed row, orchard
Next site	Go back north on Wing Levee. Right on Howard Rd. Right on Roberts Road.				
Drain @ Bowman Rd (San Joaquin Co.) Site 6	37.86267°N 121.32514°W	South on Roberts Rd to east (left) on Bowman Rd	North side of street across from "Road ends 500 feet" sign	Pump platform	alfalfa, asparagus, mixed row
Next site	Go back out Bowman to north (right) on Roberts. East (right) on Howard to Mathew Rd.				
Lone Tree Creek @ Newcastle Rd (San Joaquin Co.) Site 7	37.86220°N 121.21009°W	Mathews Rd to right on French Camp Rd. Go past Hwy 99. Left on 1st unmarked road.	SW of street near USGS gauge	West side of bridge	grapes, wheat
Next site	Continue north on Newcastle Rd to dirt road.				
Little John Creek @ Newcastle Rd (San Joaquin Co.) Site 8	37.87630°N 121.21068°W	North on Newcastle Rd on dirt road.	@ yellow gate (beware of dogs)	Off of concrete bridge	mixed field crops
Next site	Go back out of Newcastle to right on French Camp. South on Hwy 99.				

Site Name (County)	GPS Coordinates (Lat/Long,WGS84)	Directions	Parking	Sampling Location	Cropping
Walthal Slough @ Woodward Ave (San Joaquin Co.) Site 9	37.77046°N 121.29227°W	West on Hwy 120. South (left) on Airport Way. West (right) on Woodward Ave.	NE side of road @ bridge near RV park past Oakwood Lake Resort.	Off bridge	alfalfa, sod, mixed row, minimum orchard, RV camp
Next site	Go back out of Woodward Ave to south (right) on Airport Way.				
TID # 3 @ Jennings Rd (Stanislaus Co.) Site 10	37.53674°N 121.06676°W	South on Kasson Rd (J3) from Airport Way. Cross Hwy 132 and it turns into River Rd. Left on Grayson Rd (J16). South (right) on Jennings Rd.	SE of Jennings on Taylor Rd	Off of concrete slab	orchards
Next site	Continue south on Jennings Rd. Turn west (right) on Las Palmas Ave/West Main St (J17).				
Unnamed Drain @ Pomelo Ave near Paradise Ave (Stanislaus Co.) Site 11	37.46904°N 121.06274°W	From west bound J17 head south (left) on Elm Ave. Left on Pomelo Ave to nearly end of the road @ Paradise Ave.	North side of Pomelo Ave	North side of Pomelo Ave	alfalfa, mixed row
Next site	Return to laboratory: Go back out on Pomelo Ave to Hwy 33.				

Table 2. Sample locations in the Sacramento River watershed

Site Name (County)	GPS Coordinates (Lat/Long,WGS84)	Directions	Parking	Sampling Location	Cropping
Drain @ Midway Rd east of Pedrick Rd and west of Robben Rd (Solano Co.) Site 12	38.41648°N 121.79452°W	I-80 to South on Pedrick Rd. East (left) on Midway Rd.	~0.5 miles from Pedrick on north side of Midway Rd.	North side of Midway Rd. Large drain that runs perpendicular to the road	alfalfa, mixed row, grapes
Next site	Go back out on Midway to a left on Pedrick.				
Drain @ Ulatis Creek @ Hwy 113 (Solano Co.) Site 13	38.33838°N 121.82330°W	South on Hwy 113	Turnout on west side of Hwy 113 just before Ulatis Creek bridge.	At culvert entering @ Ulatis Creek	alfalfa, mixed row
Next site	Go back North on Hwy 113. West (left) on Midway Rd.				
Drain @ Midway Rd west of Schoeder/Batavia Rd (Solano Co.) Site 14	38.41680°N 121.87325°W	Hwy 113 to west (left) on Midway Rd. ~0.6 miles west of Batavia Rd	South side of Midway.	Off downstream side of bridge.	alfalfa, mixed row
Next site	Continue west on Midway past I-80 to north on I-505.				
Lateral to Gordon Slough @ Rd 19 (Yolo Co.) Site 15	38.71881°N 121.95438°W	I-505 to east on Rd 19	South side of Rd 19 on gravel concrete bridge just off exit	Off concrete bridge south of Rd 19	alfalfa, mixed row, grazing
Next site	Continue east on Rd 19.				

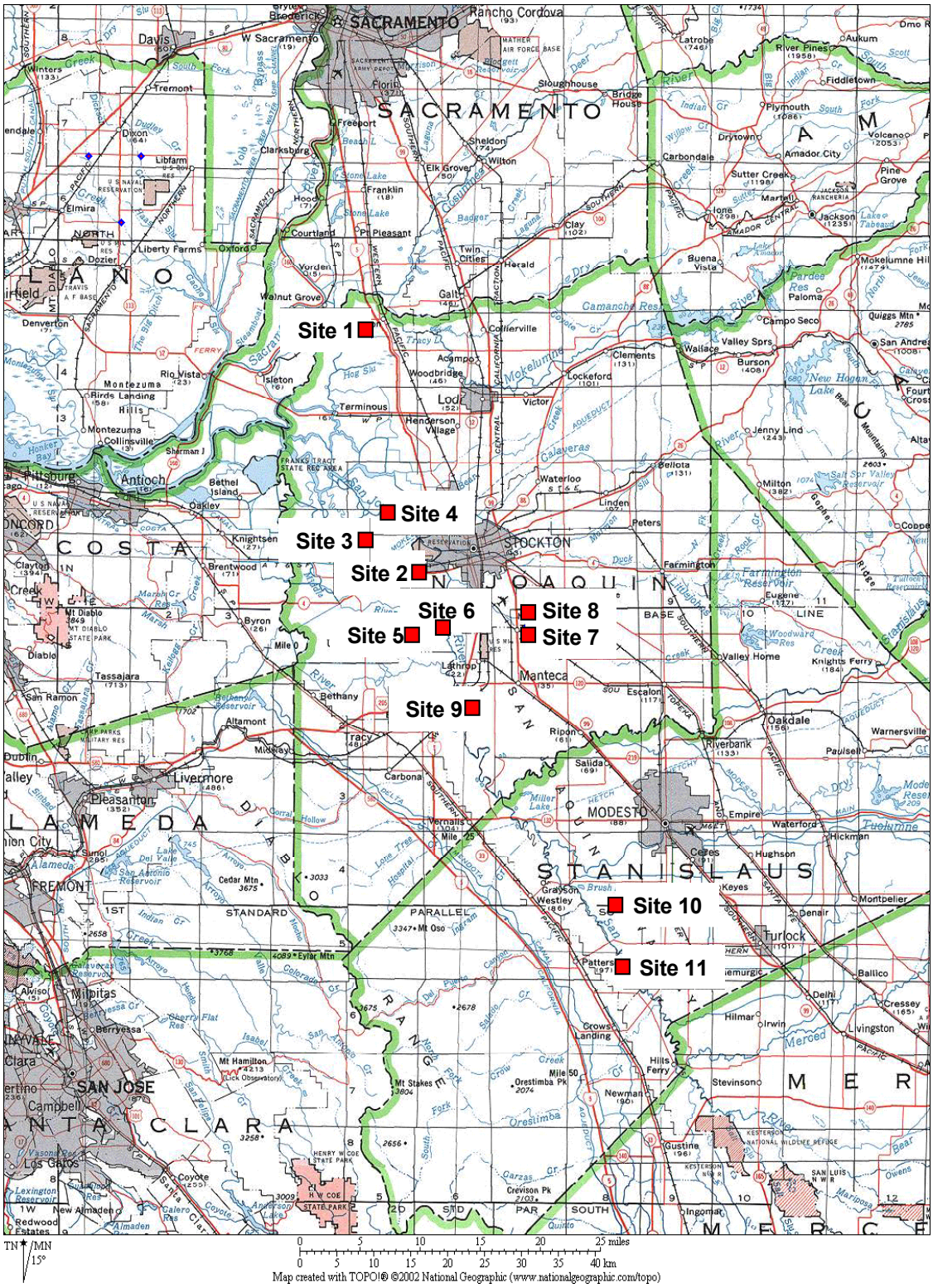
Site Name (County)	GPS Coordinates (Lat/Long,WGS84)	Directions	Parking	Sampling Location	Cropping
Gordon Slough @ Rd 19 (Yolo Co.) Site 16	38.71465°N 121.92439°W	East on Rd 19	~1.5 miles from I-505 south of Rd 19 on dirt road on concrete bridge	East side of bridge off of road (very narrow)	alfalfa, mixed row, grazing
Next site	Continue east on Rd 19. Turn south (right) on Rd 94B. East (left) on Rd 24 to south (right) on Rd 95. East (left) on Rd 27.				
Willow Slough @ Rd 27 (Yolo Co.) Site 17	38.61960°N 121.83234°W	Rd 27 to bridge east of Rd 96	At gate on north east side of bridge.	Off downstream side of bridge	alfalfa, mixed row
Next site	Go east on Rd 27 to Hwy 113 or west to I-505				
Stone Corral Creek @ 4 Mile Rd (Colusa Co.) Site 18	39.29337°N 122.11665°W	I-5 north of Williams to Maxwell Rd east. Turn north (left) on 4 Mile Rd.	~1.2 miles north of Maxwell @ steel guardrail SW side of bridge on dirt road	Off downstream side of bridge	rice
Next site	Continue north on 4 Mile Rd.				
East Drain @ 4 Mile Rd (Colusa Co.) Site 19	39.30535°N 121.11652°W	North on 4 Mile Rd to concrete bridge	On dirt road north of concrete bridge	Off downstream side of bridge	rice
Next site	Go back south on 4 Mile to west on Maxwell to south on I-5.				
Elk Creek @ Hahn & Miller Rd's (Colusa Co.) Site 20	39.05663°N 122.02300°W	I-5 to east on Hahn Rd to Miller Rd	North side of bridge on Miller	Off downstream side of bridge	mixed row

Next site	Continue north on Miller Rd.				
Site Name (County)	**GPS Coordinates (Lat/Long,WGS84)**	**Directions**	**Parking**	**Sampling Location**	**Cropping**
Sand Creek @ Miller Rd (Colusa Co.) Site 21	39.06779°N 122.02279°W	North on Miller Rd	~0.8 miles north of Hahn Rd southeast of bridge on dirt road	Off downstream side of bridge	mixed row
Next site	Go back south on Miller to west on Hahn to south on I-5.				
Drain south of Road 14 (Yolo Co.) Site 22	38.77894°N 121.81824°W	I-5 to east @ Zamora exit Rd 13 (E10). South (right) on Rd 97 to east (left) on Rd 14	1st bridge east of Rd 97 on southeast side of bridge	Off north side of bridge	mixed row
Next site	Continue east on Rd 14 to south on Rd 102.				
Knight's Landing Ridge Cut @ Rd 16 South (Yolo Co.) Site 23	38.74842°N 121.69489°W	Rd 102 to east (left) on Rd 16	Next to gate on either side of the street	Off south pump platform	alfalfa, tomatoes cotton
Next site	Cross the street to the other pump.				
Knight's Landing Ridge Cut @ Rd 16 North (Yolo Co.) Site 24	38.74894°N 121.69498°W	Rd 102 to east (left) on Rd 16	Next to gate on either side of the street	Off north pump platform	alfalfa, tomatoes cotton
Next site	Return to laboratory: Go back west on Rd 16 to south on Rd 102. Turn west (left) on Rd 17 to Hwy 113 south.				

APPENDIX B

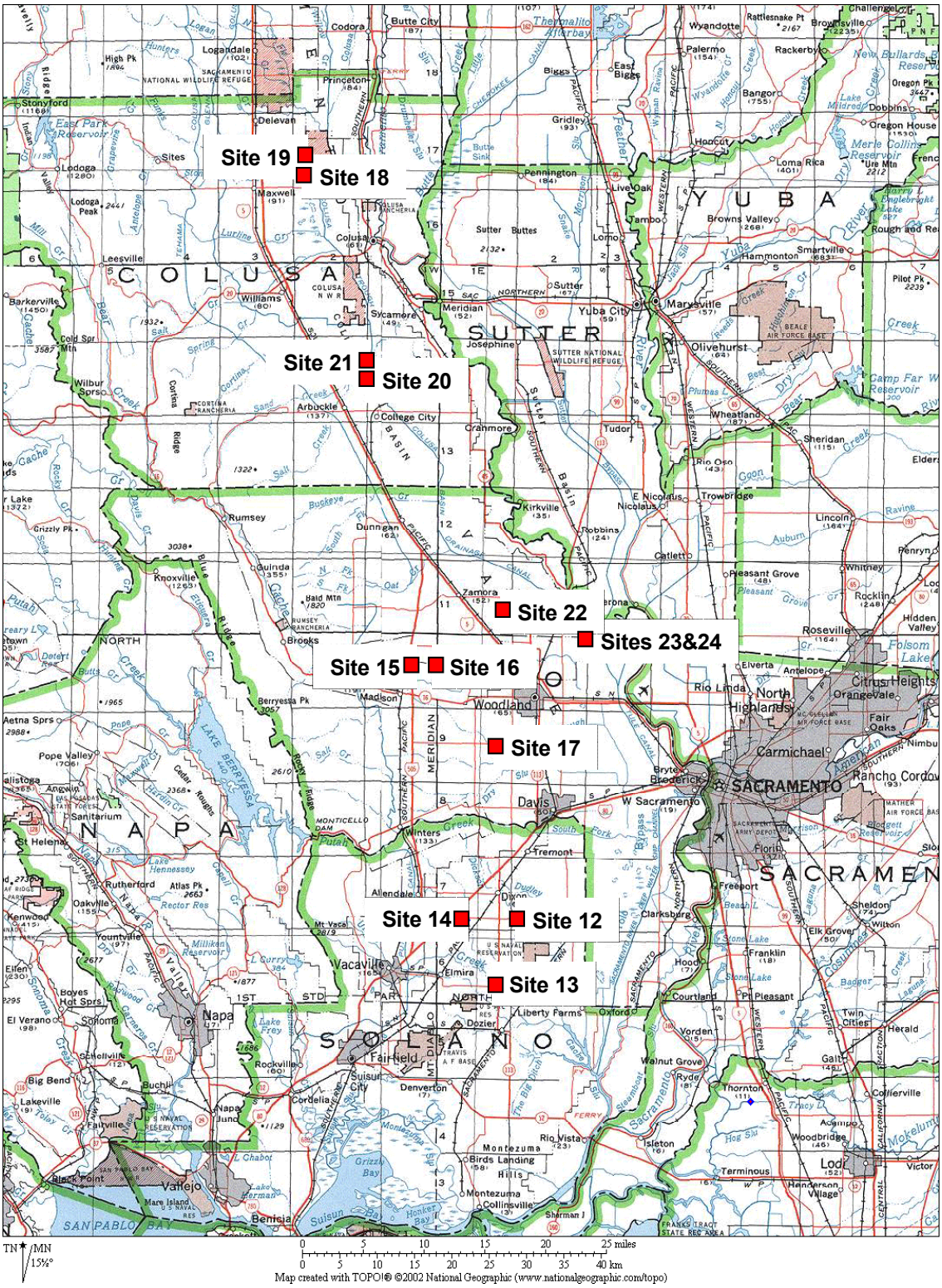
Site Maps

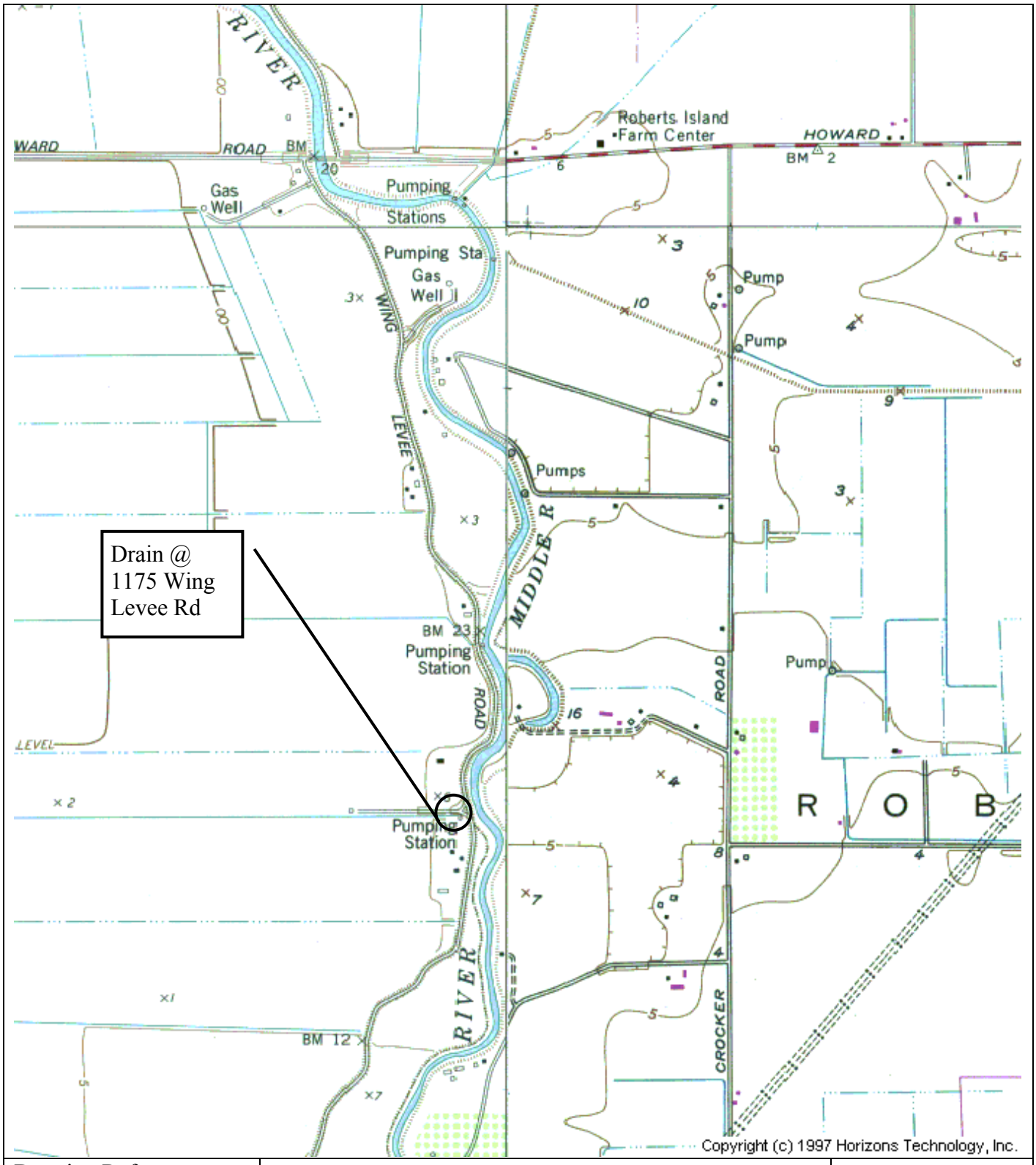
Sample locations in the San Joaquin River/Delta watersheds



Investigations of Water Quality in Agricultural Drains of the Central Valley

Sample locations in the Sacramento River watershed





Drawing Reference:

U.S.G.S
TOPOGRAPHIC MAP

7.5 MINUTE QUAD

Photorevised 2003

SITE LOCATION MAP

Drain @ 11751 Levee Rd

SAN JOAQUIN COUNTY

37.85659 N 121.37801 W

N
W
E
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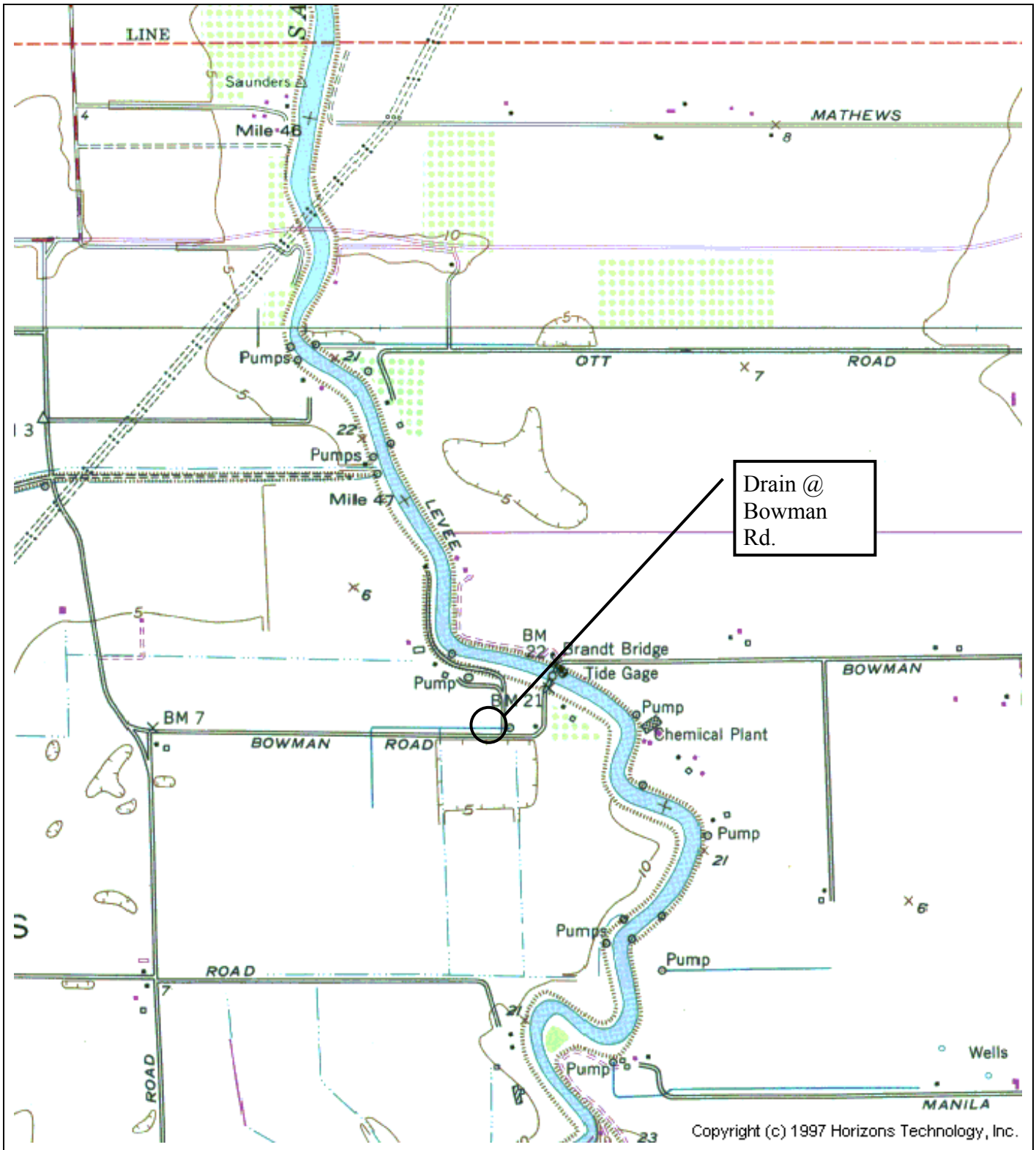
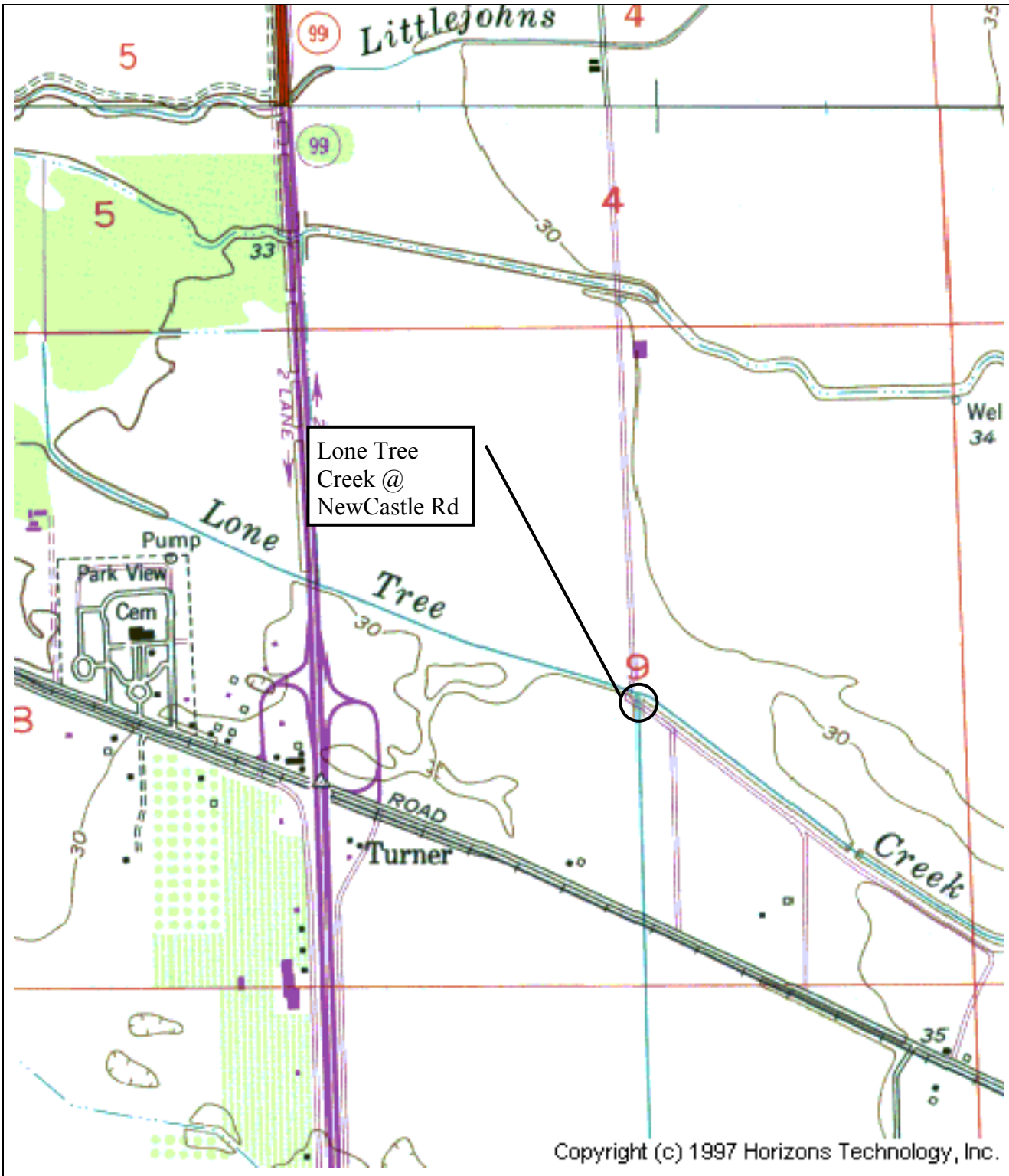
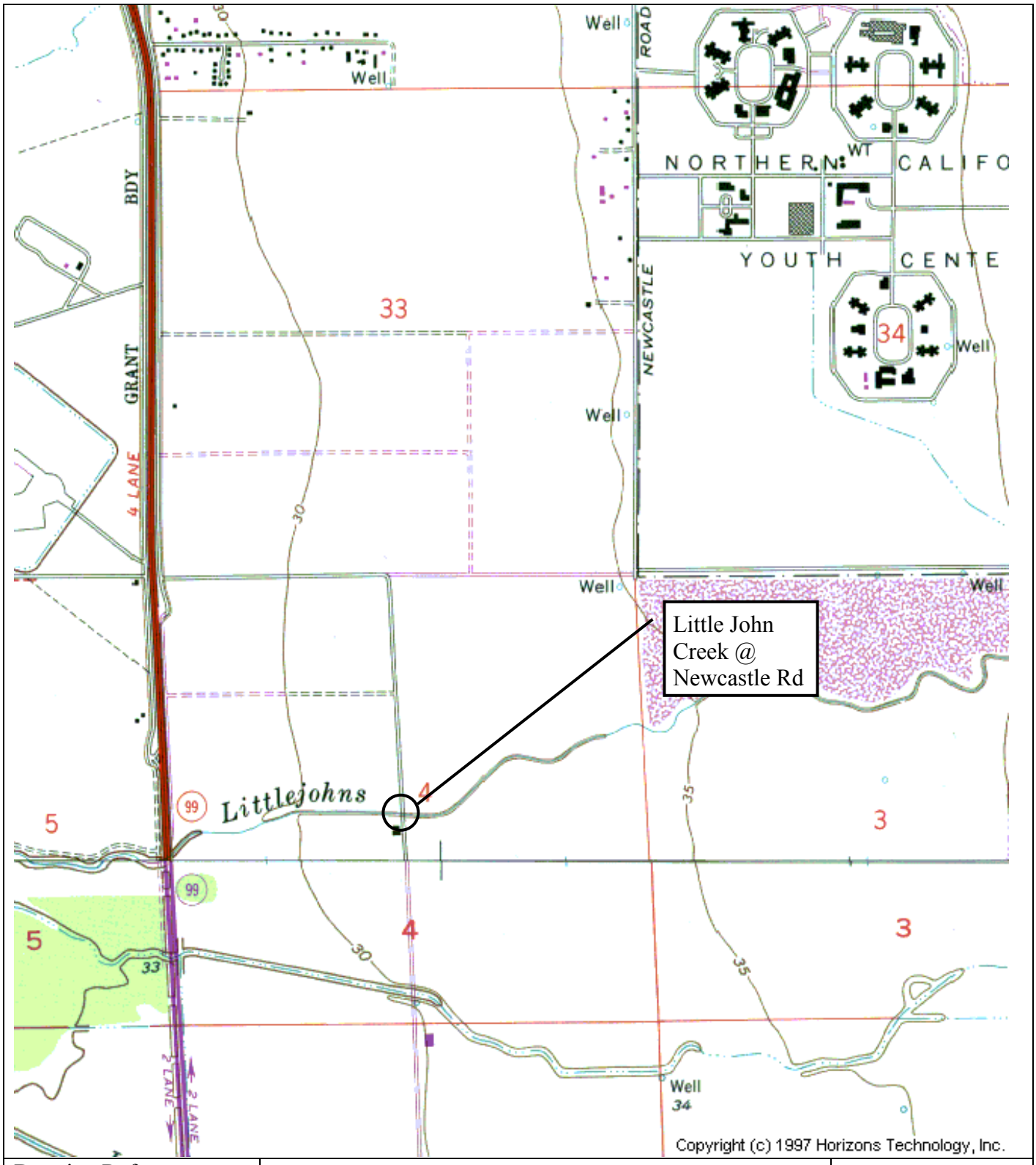
approx. scale



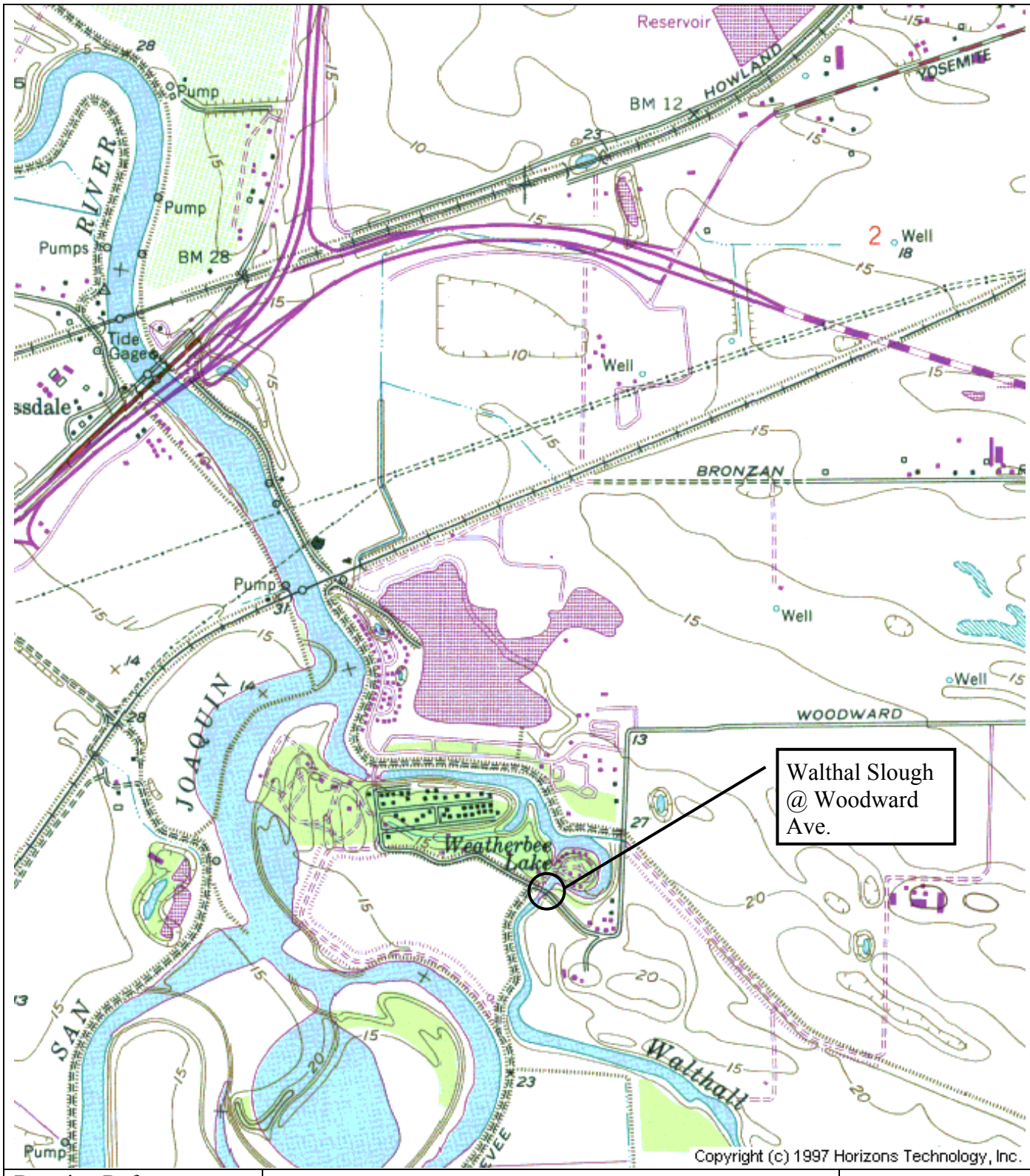
Drawing Reference: U.S.G.S TOPOGRAPHIC MAP 7.5 MINUTE QUAD Photorevised 2003	**SITE LOCATION MAP** Drain @ Bowman Rd. **SAN JOAQUIN COUNTY** 37.86267 N 121.32514 W	N W E S approx. scale



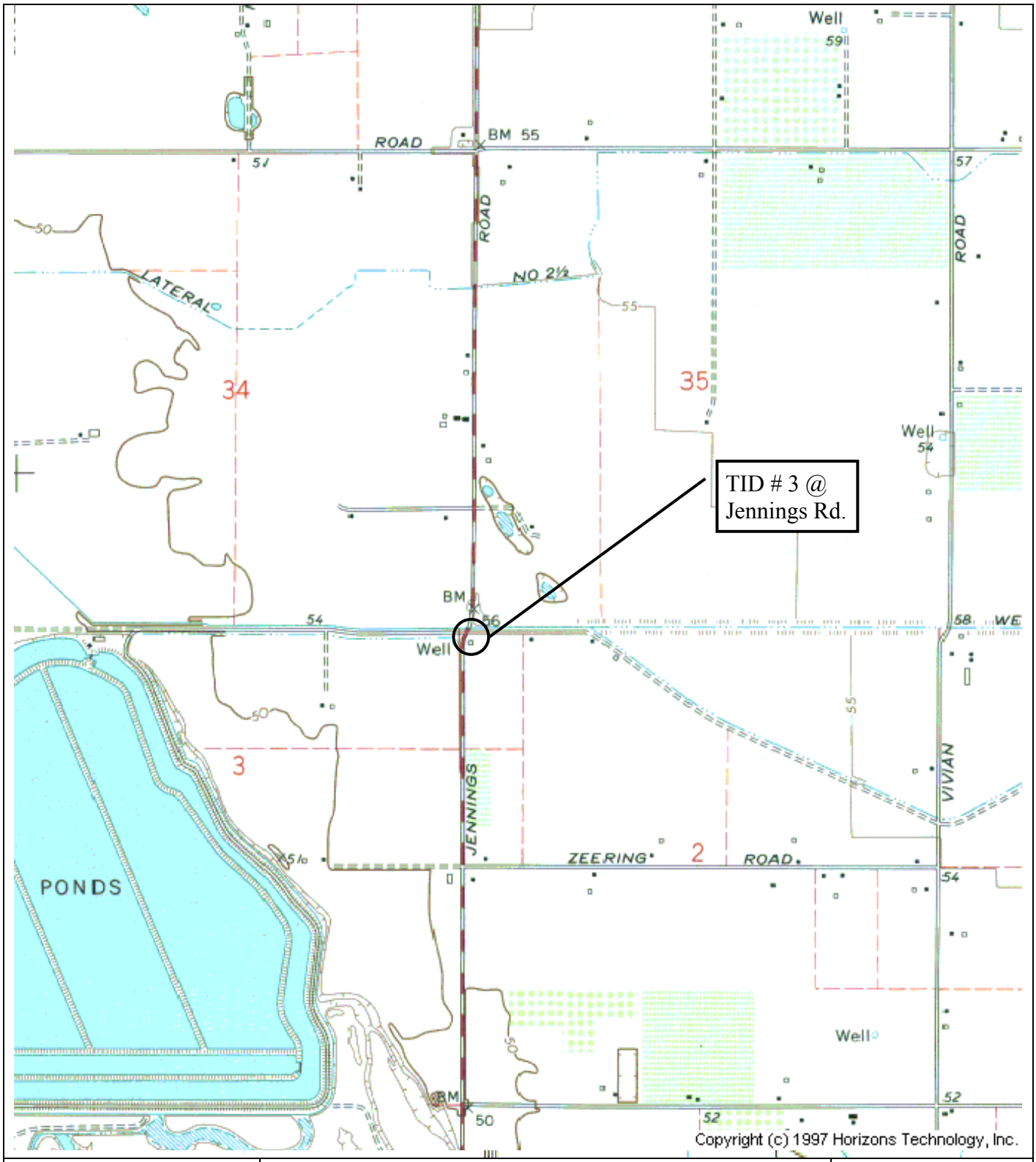
Drawing Reference: U.S.G.S TOPOGRAPHIC MAP 7.5 MINUTE QUAD Photorevised 2003	**SITE LOCATION MAP** Lone Tree Creek @ Newcastle Rd. **SAN JOAQUIN COUNTY** 37.86220 N 121.21009 W	N W E S approx. scale



Drawing Reference: U.S.G.S TOPOGRAPHIC MAP 7.5 MINUTE QUAD Photorevised 2003	**SITE LOCATION MAP** Little John Creek @ Newcastle Rd. **SAN JOAQUIN COUNTY** 37.87630 N 121.21068 W	N W E S approx. scale



Drawing Reference: U.S.G.S TOPOGRAPHIC MAP 7.5 MINUTE QUAD Photorevised 2003	**SITE LOCATION MAP** Walthal Slough @ Woodward Ave. **SAN JOAQUIN COUNTY** 37.77046 N 121.29227 W	N W E S approx. scale



Drawing Reference:

U.S.G.S TOPOGRAPHIC MAP

7.5 MINUTE QUAD

Photorevised 2003

SITE LOCATION MAP

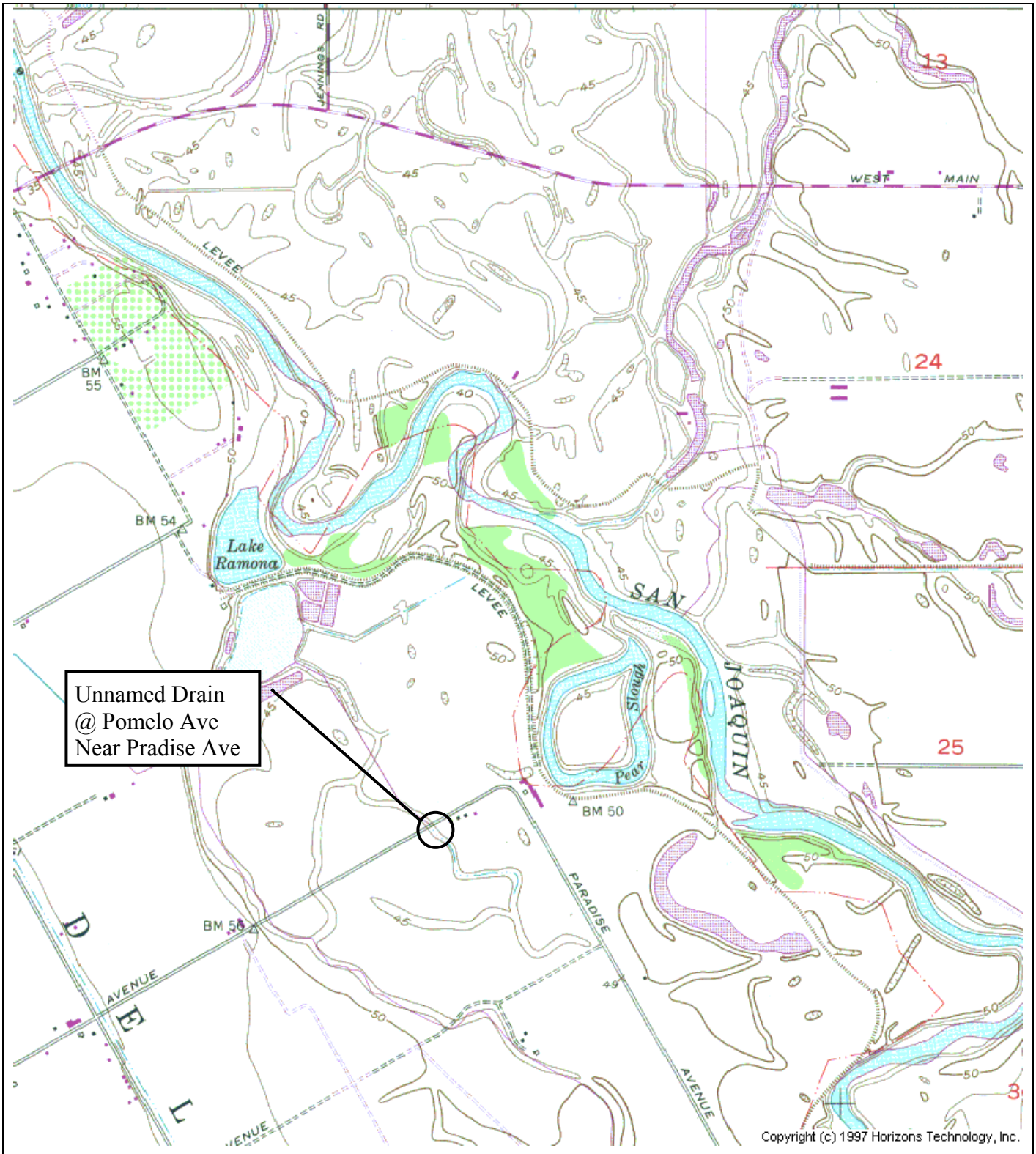
TID # 3 @ Jennings Rd.

STANISALUS COUNTY

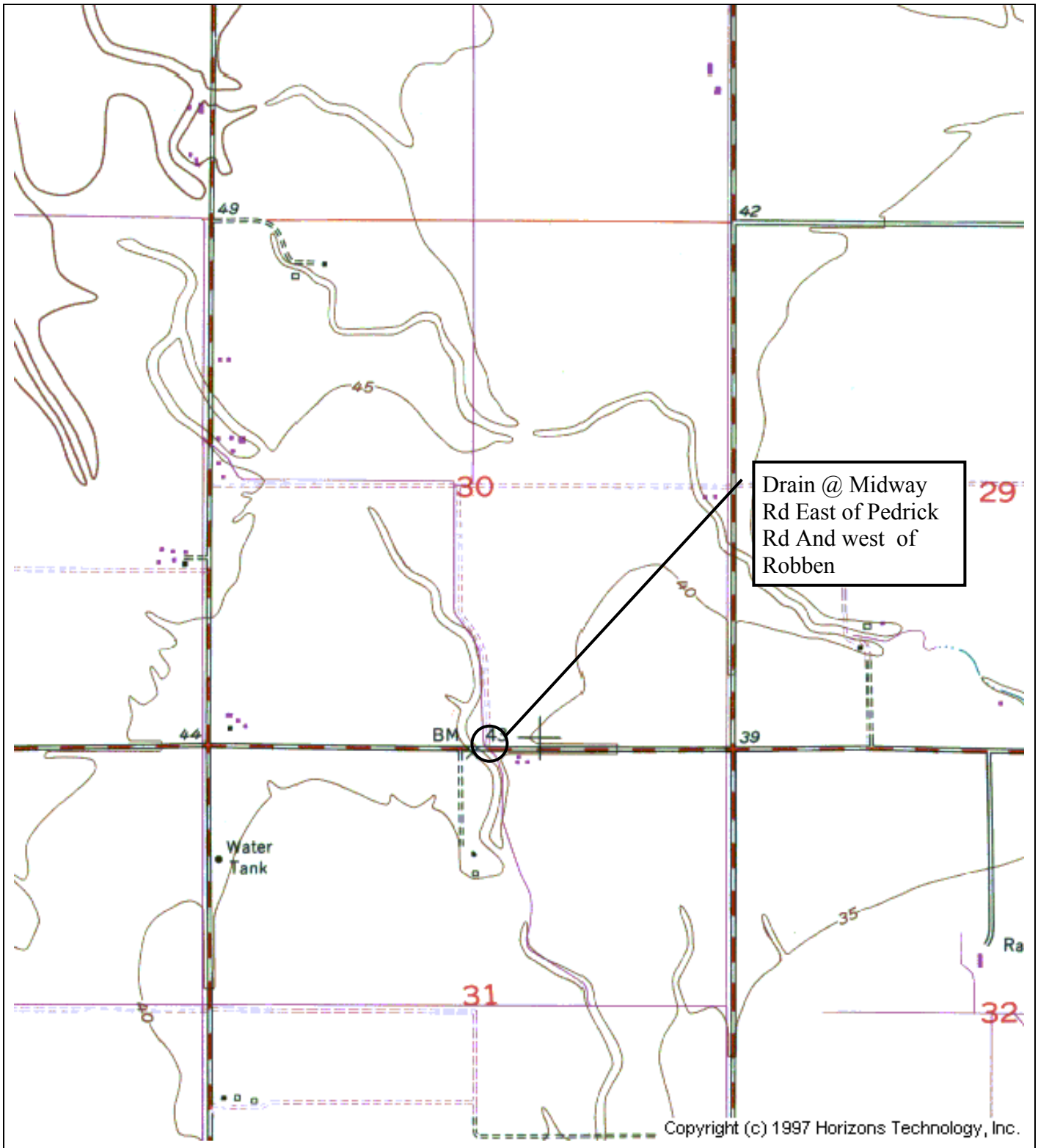
37.53674 N 121.06676 W

N
W E
S

approx. scale



Drawing Reference: U.S.G.S TOPOGRAPHIC MAP 7.5 MINUTE QUAD Photorevised 2003	**SITE LOCATION MAP** Unnamed Drain @ Pomelo Ave near Paradise Ave **STANISLAUS COUNTY** 37.46904 N 121.06274 W	N W E S approx. scale



Drawing Reference:

U.S.G.S TOPOGRAPHIC MAP

7.5 MINUTE QUAD

Photorevised 2003

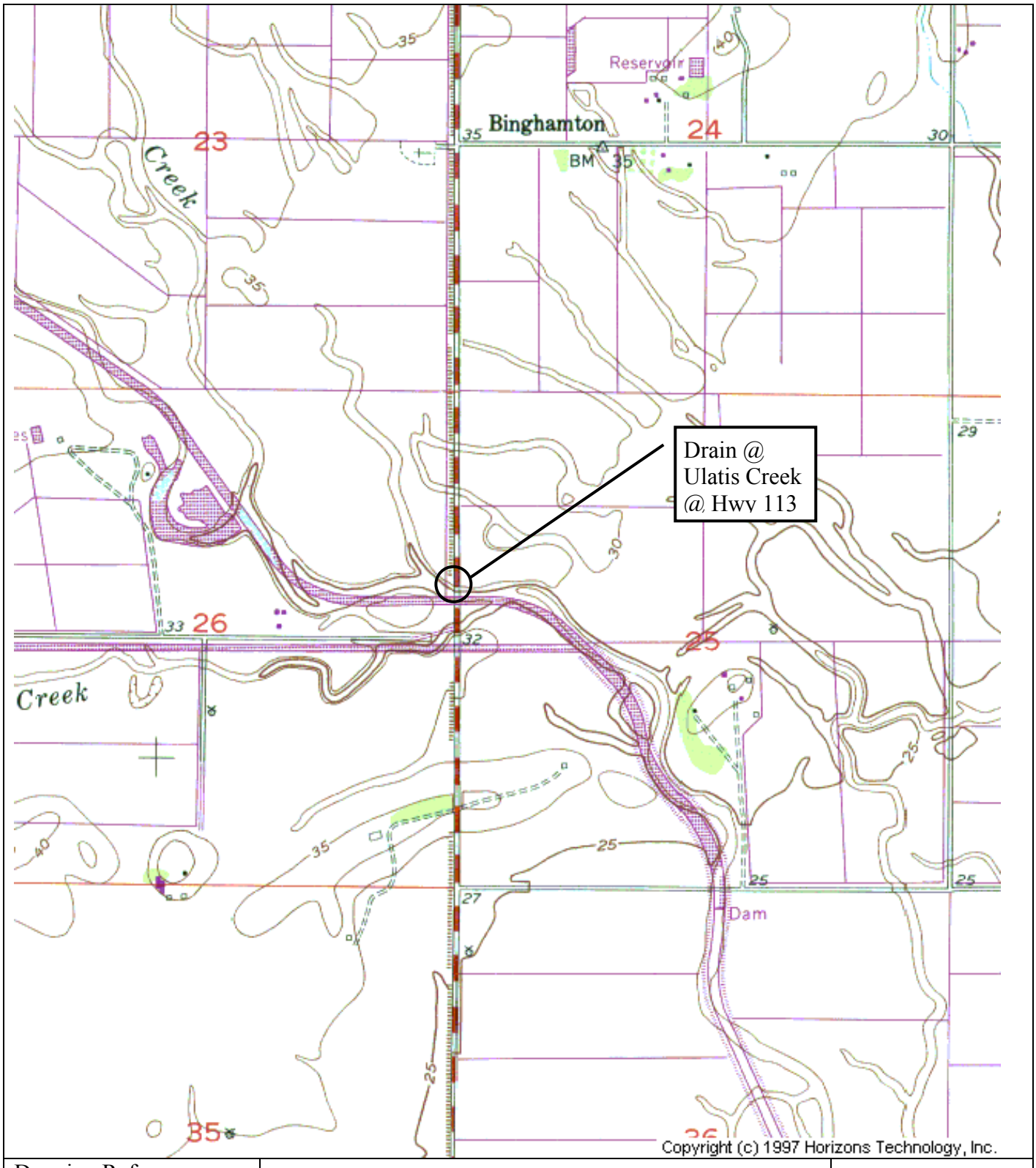
SITE LOCATION MAP

Drain @ Midway Rd east of pedrick Rd and west of Robben Rd

SOLANO COUNTY

38.41648 N 121.79452 W

approx. scale



Drawing Reference:

U.S.G.S
TOPOGRAPHIC MAP

7.5 MINUTE QUAD

Photorevised 2003

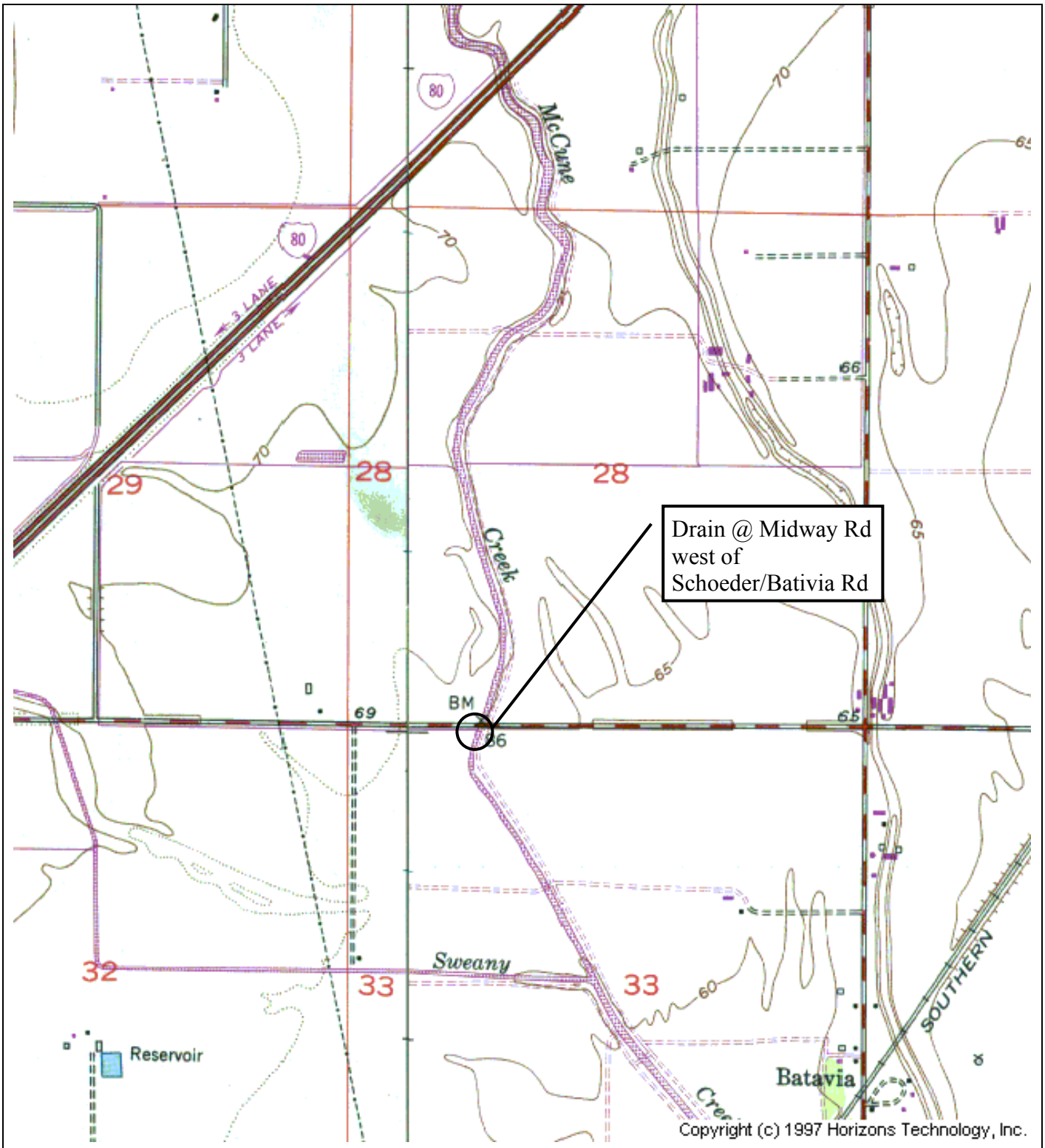
SITE LOCATION MAP

Drain @ Ulatis Creek @ Hwy 113

SOLANO COUNTY

38.33838 N 121.82330 W

N
W
E
S
approx. scale



Drawing Reference:

U.S.G.S TOPOGRAPHIC MAP

7.5 MINUTE QUAD

Photorevised 2003

SITE LOCATION MAP

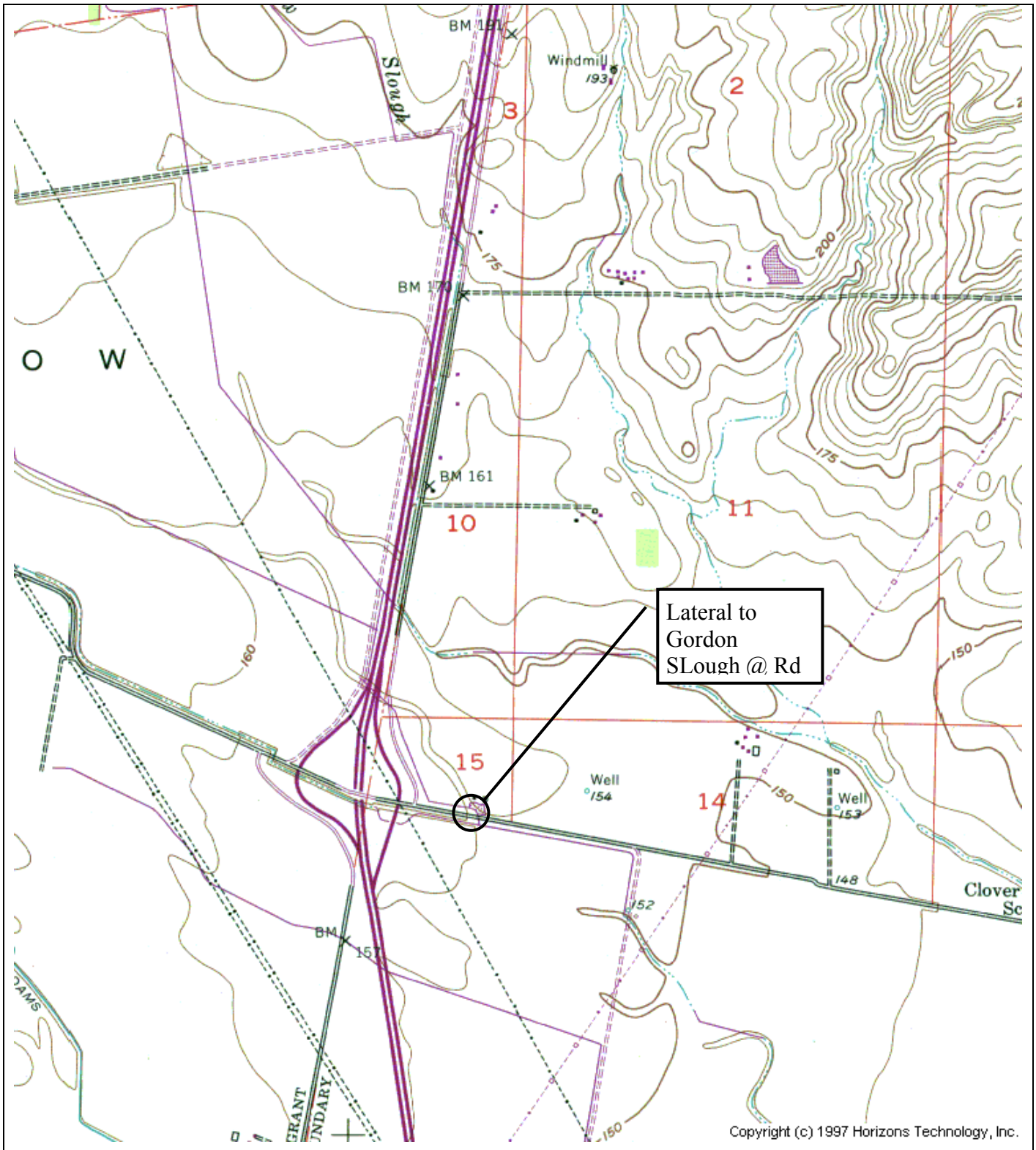
Drain @ Midway Rd west of Schoeder/Bativia Rd

SOLANO COUNTY

38.41680 N 121.87325 W

N W E S

approx. scale



Drawing Reference:

U.S.G.S TOPOGRAPHIC MAP

7.5 MINUTE QUAD

Photorevised 2003

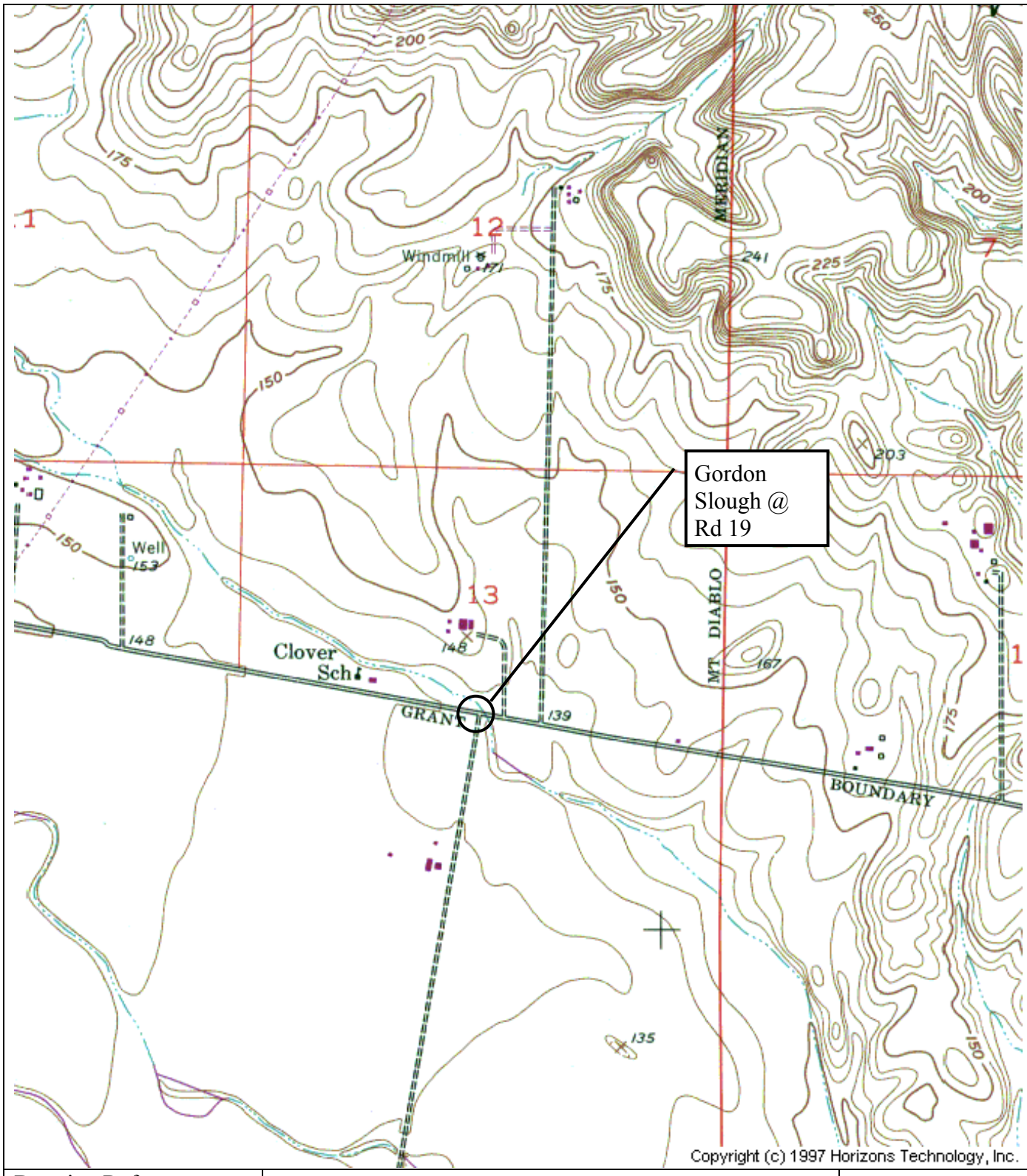
SITE LOCATION MAP

Lateral to Gordon Slough @ Rd 19

YOLO COUNTY

38.71881 N 121.95438 W

approx. scale



Drawing Reference:

U.S.G.S
TOPOGRAPHIC MAP

7.5 MINUTE QUAD

Photorevised 2003

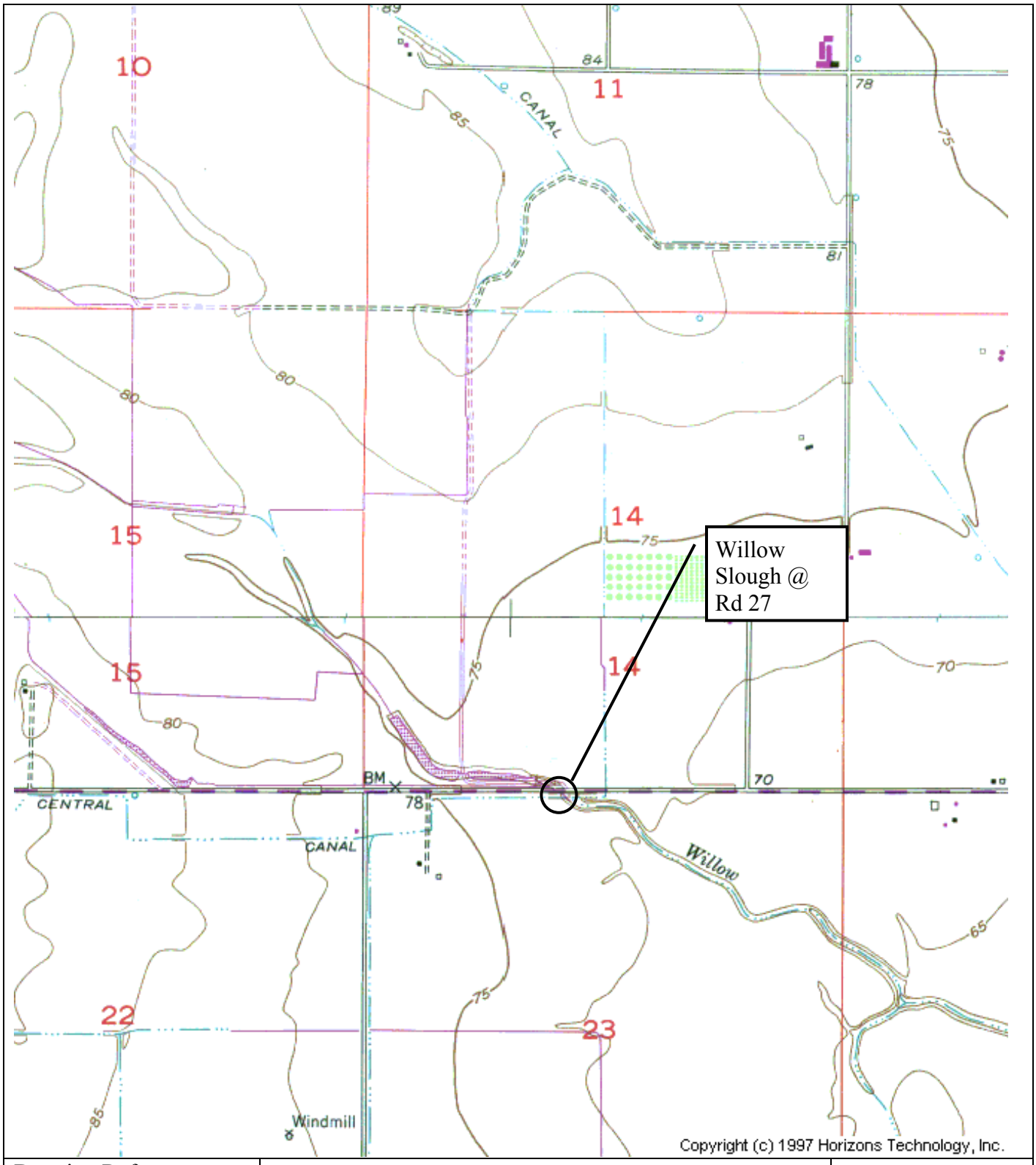
SITE LOCATION MAP

Gordon Slough @ Rd 19

YOLO COUNTY

38.71465 N 121.92439 W

approx. scale



Drawing Reference:

U.S.G.S
TOPOGRAPHIC MAP

7.5 MINUTE QUAD

Photorevised 2003

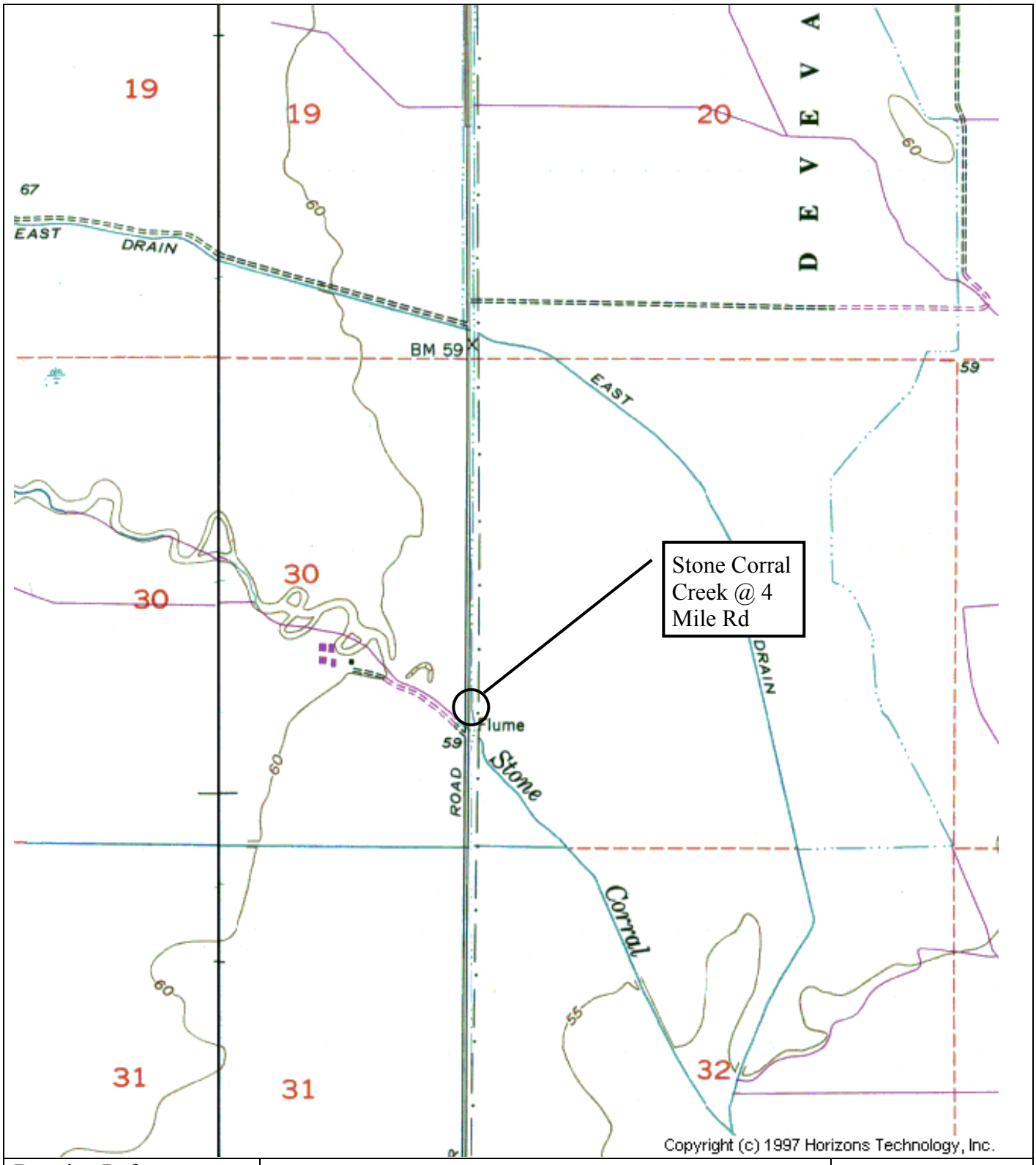
SITE LOCATION MAP

Willow Slough @ Rd 27

YOLO COUNTY

38.61960 N 121.83234 W

approx. scale.



Drawing Reference:

U.S.G.S TOPOGRAPHIC MAP

7.5 MINUTE QUAD

Photorevised 2003

SITE LOCATION MAP

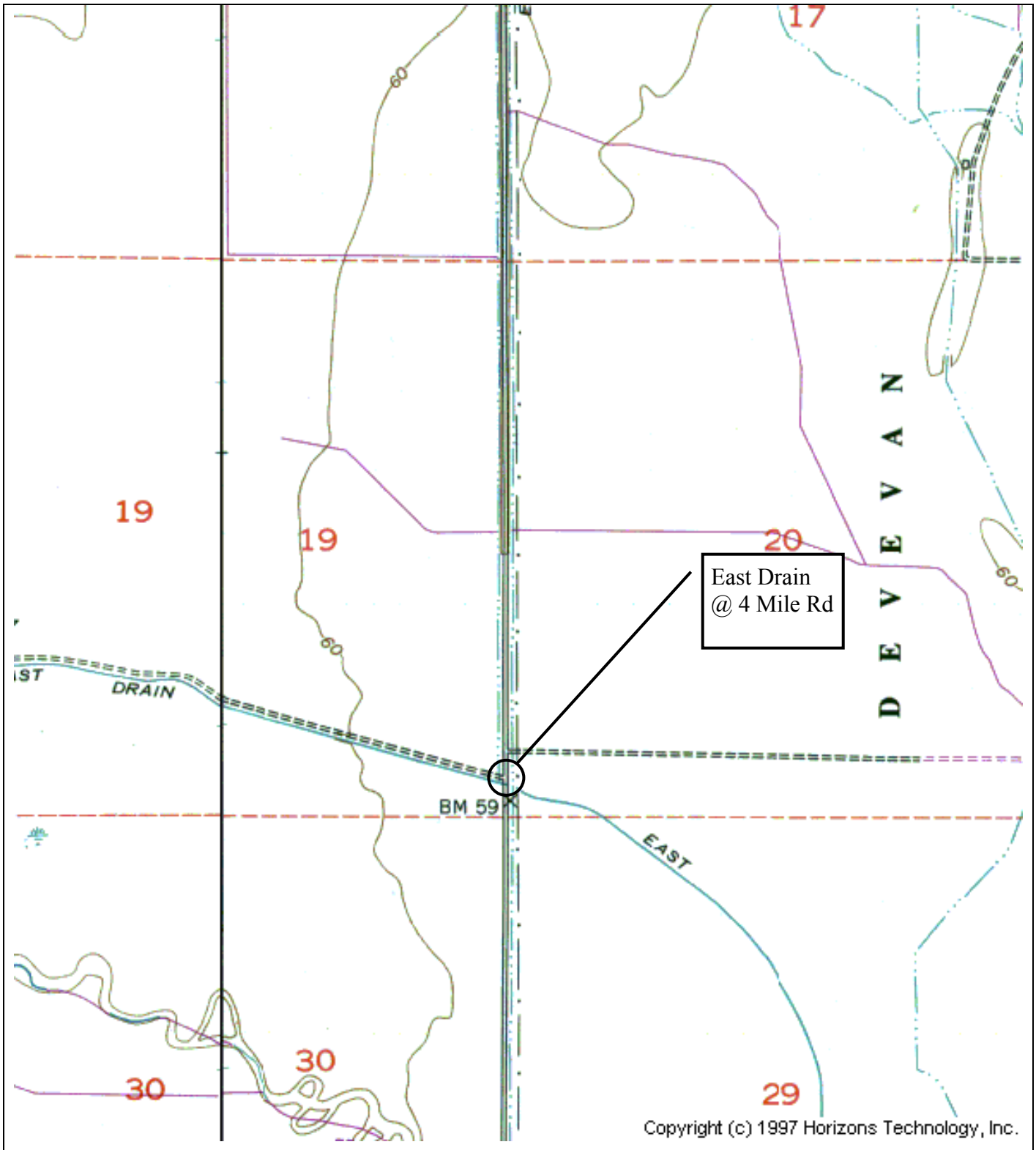
Stone Corral Creek @ 4 Mile Rd

COLUSA COUNTY

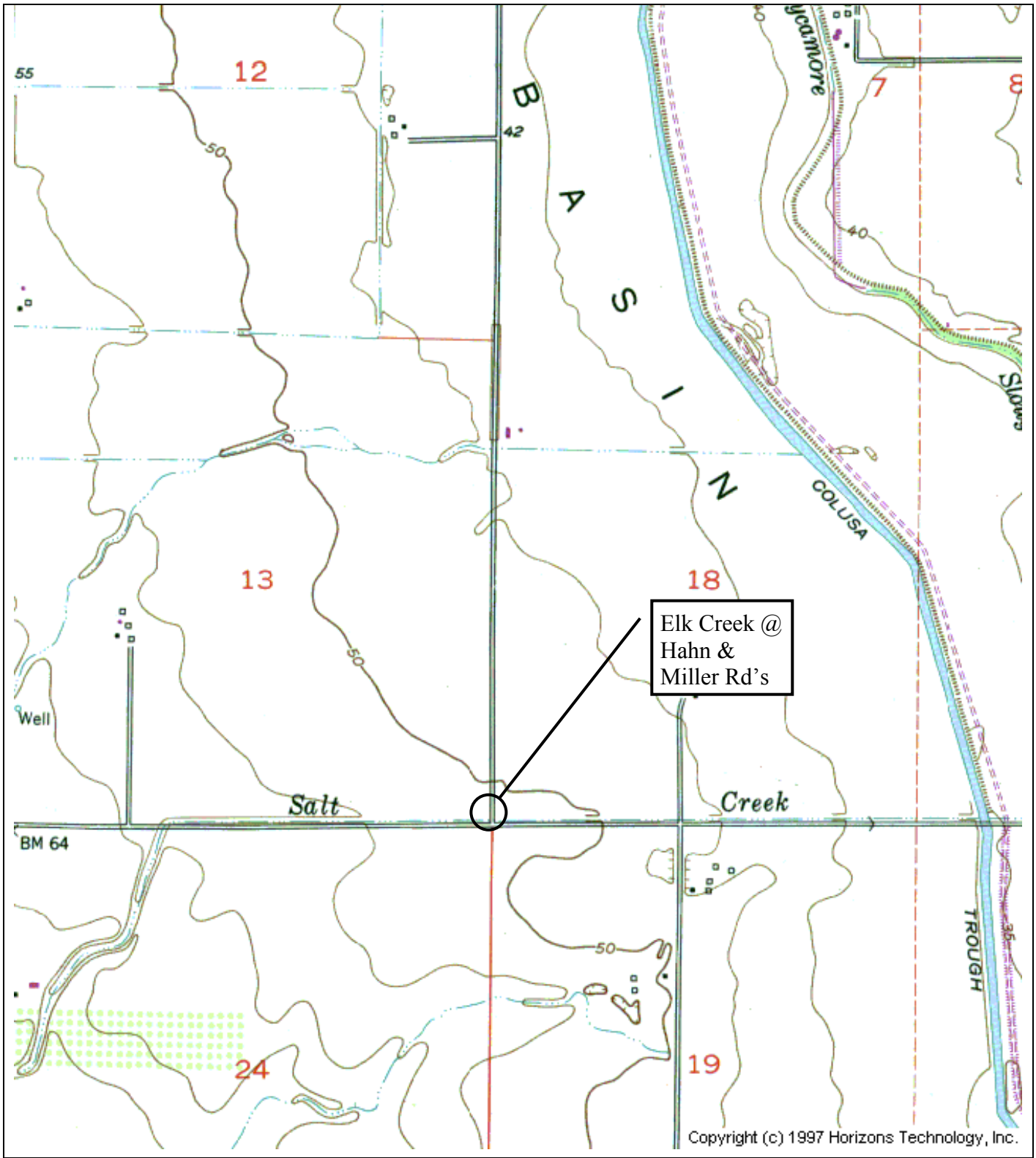
39.29337 N 122.11665 W

N
W E
S

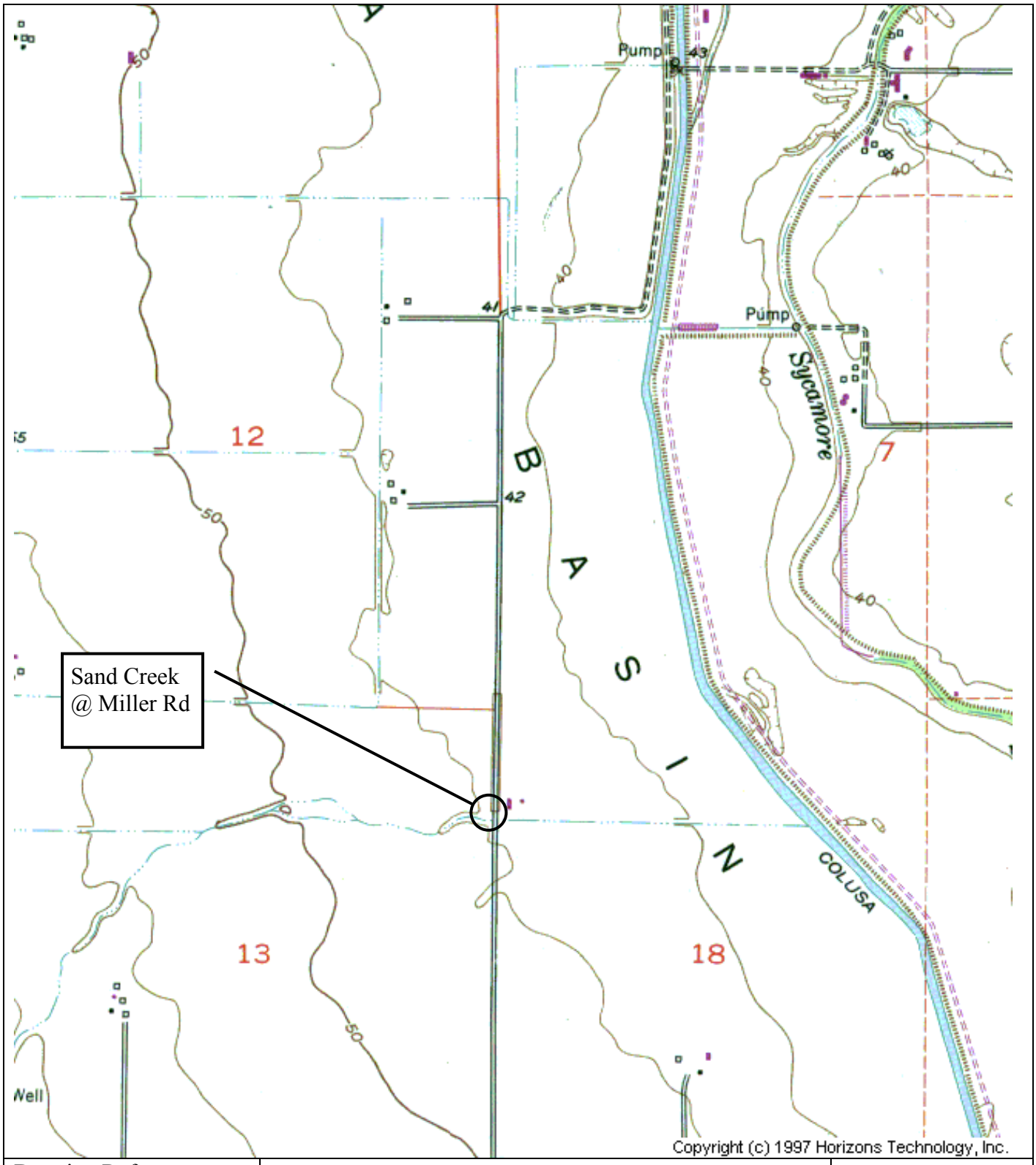
approx. scale



Drawing Reference: U.S.G.S TOPOGRAPHIC MAP 7.5 MINUTE QUAD Photorevised 2003	**SITE LOCATION MAP** East Drain @ 4 Mile Rd **COLUSA COUNTY** 39.30535 N 122.11652 W	N W E S approx. scale



Drawing Reference: U.S.G.S TOPOGRAPHIC MAP 7.5 MINUTE QUAD Photorevised 2003	**SITE LOCATION MAP** Elk Creek @ Hahn & Miller Rd's **COLUSA COUNTY** 39.05663 N 122.02300 W	N W E S approx. scale



Drawing Reference:

U.S.G.S
TOPOGRAPHIC MAP

7.5 MINUTE QUAD

Photorevised 2003

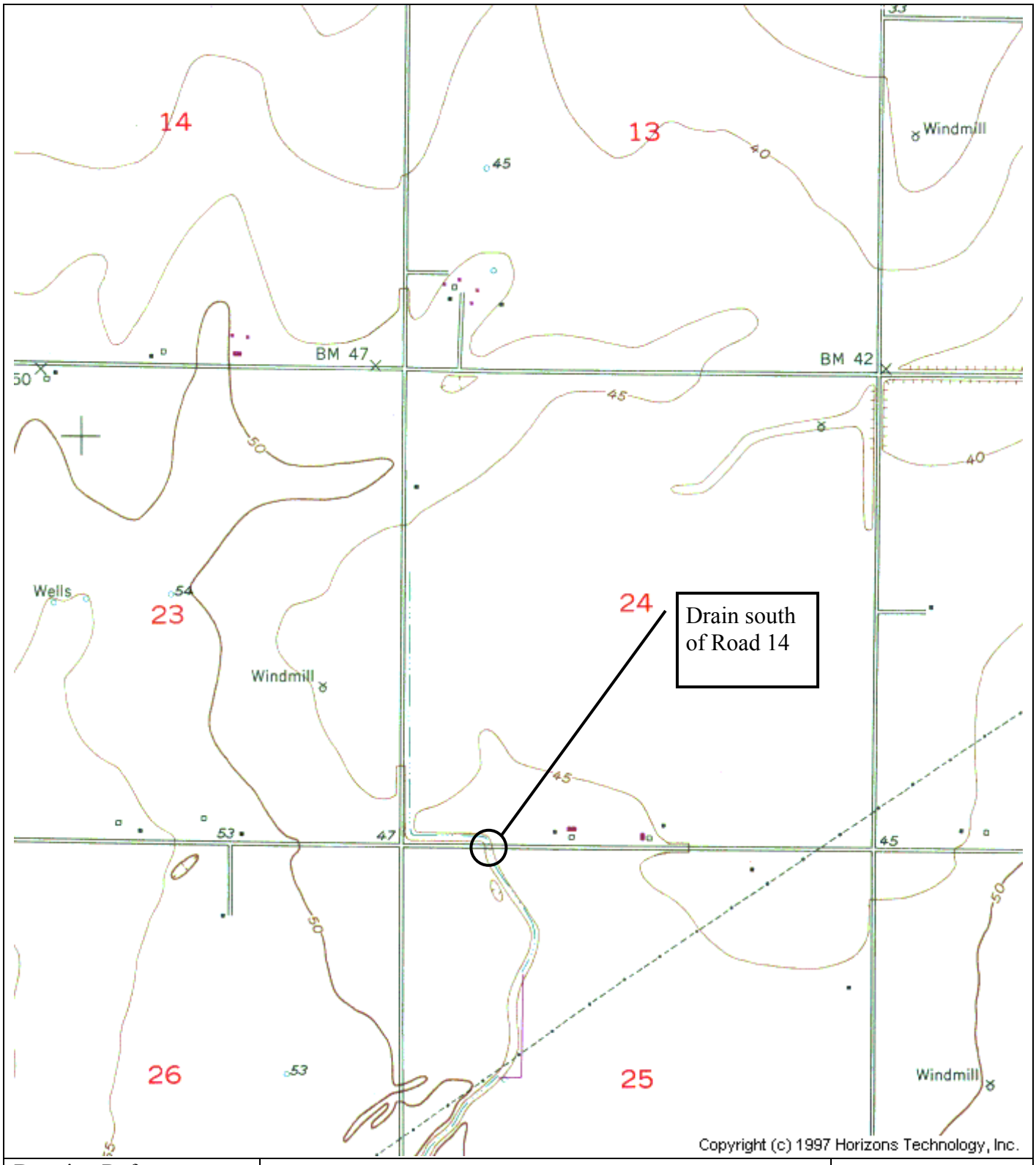
SITE LOCATION MAP

Sand Creek @ Miller Rd

COLUSA COUNTY

39.06779 N 122.02279 W

N
W
E
S
approx. scale



Drawing Reference:

U.S.G.S
TOPOGRAPHIC MAP

7.5 MINUTE QUAD

Photorevised 2003

SITE LOCATION MAP

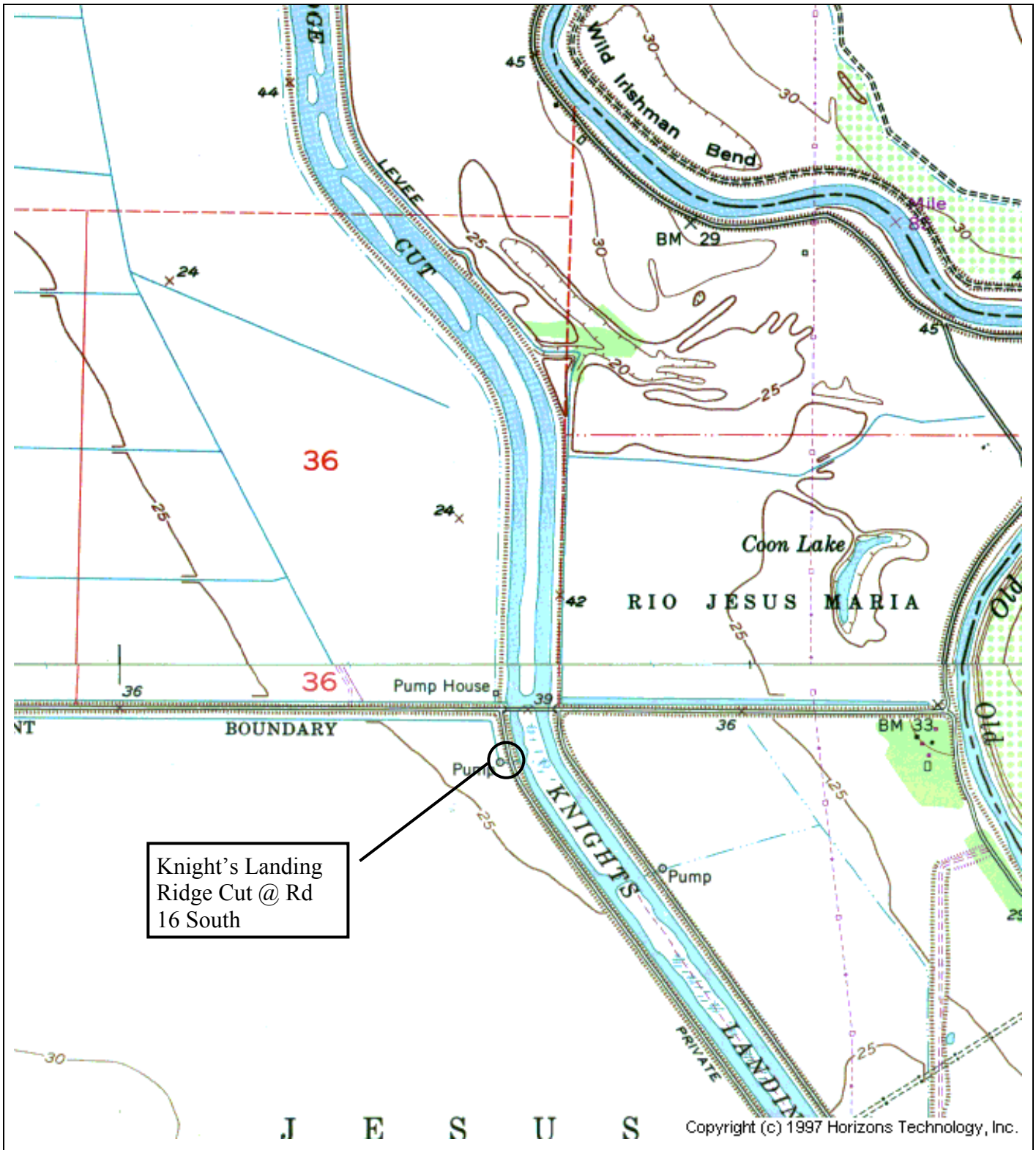
Drain south of Road 14

YOLO COUNTY

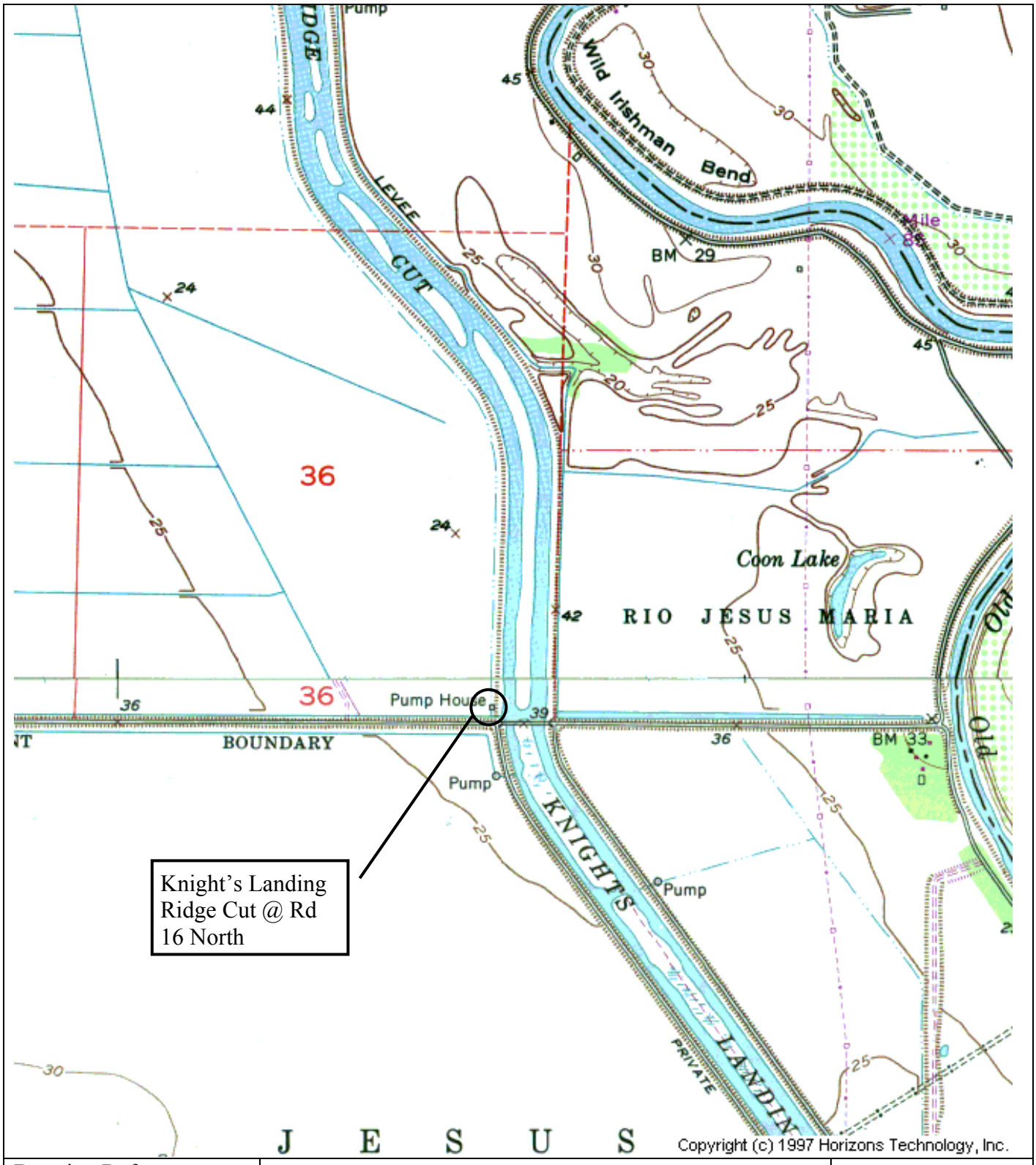
38.77894 N 121.81824 W

N
W E
S

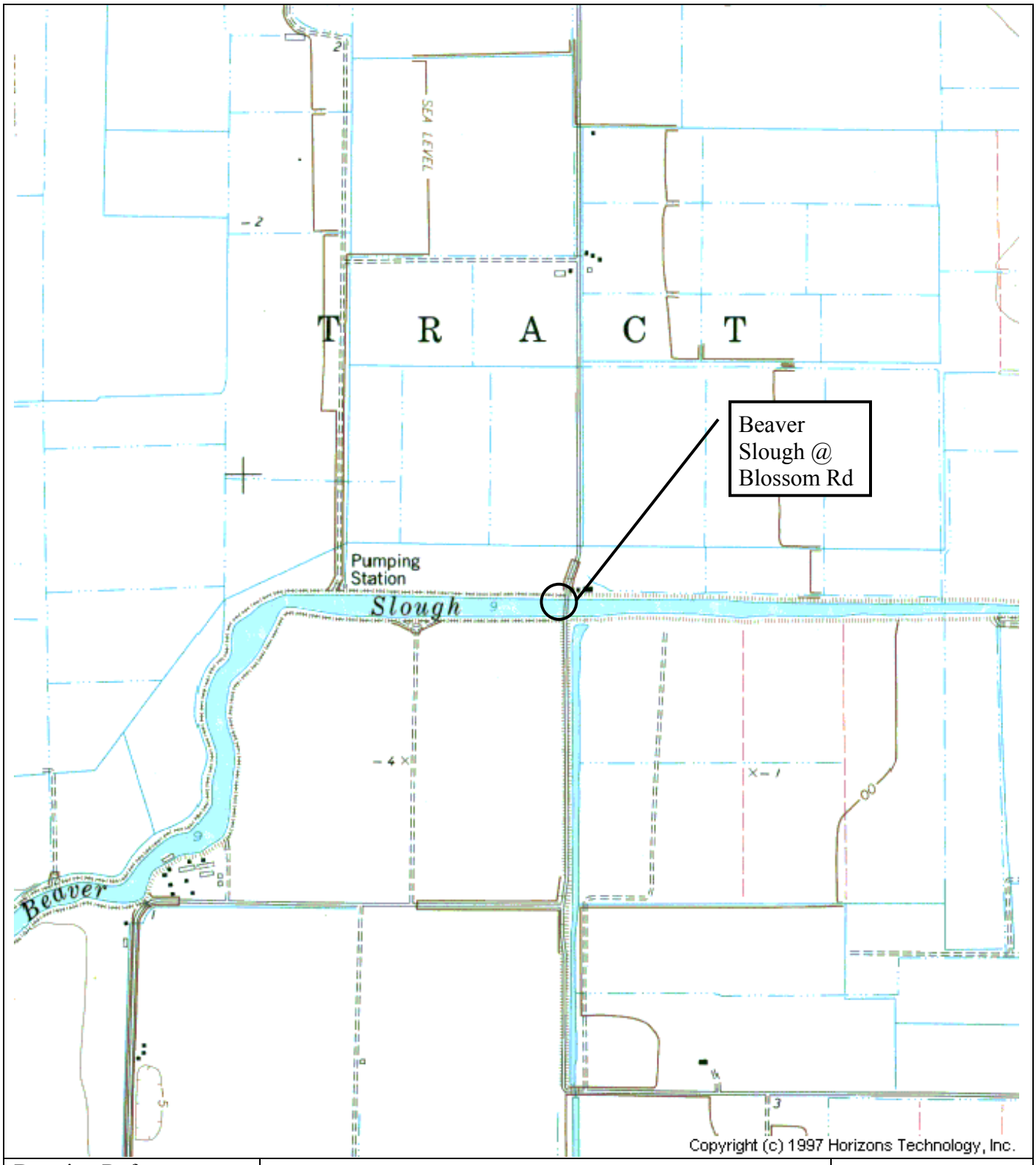
approx. scale



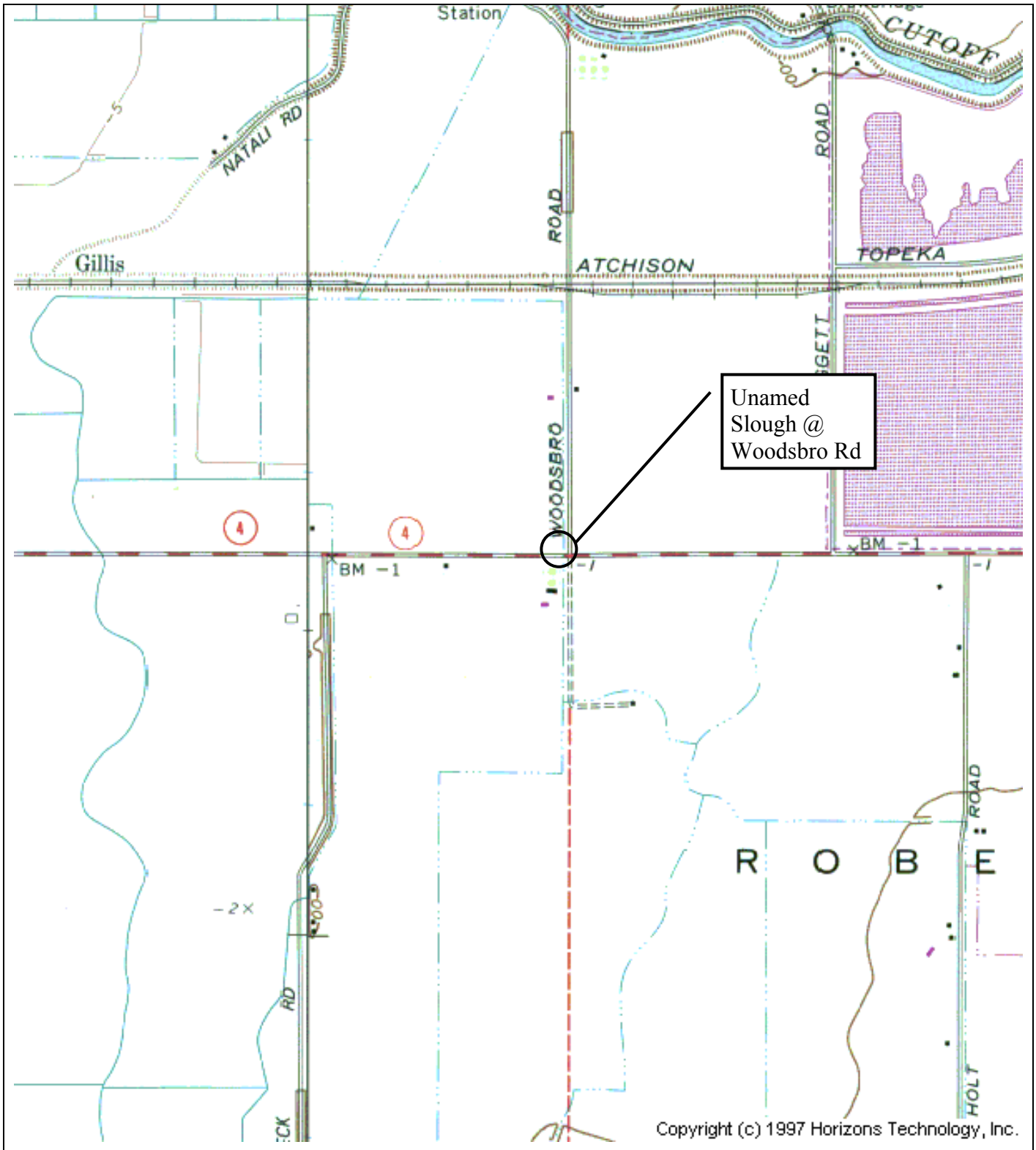
Drawing Reference: U.S.G.S TOPOGRAPHIC MAP 7.5 MINUTE QUAD Photorevised 2003	**SITE LOCATION MAP** Knight's Landing Ridge Cut @ Rd 16 South **YOLO COUNTY** 38.74842 N 121.69489 W	N W E S approx. scale



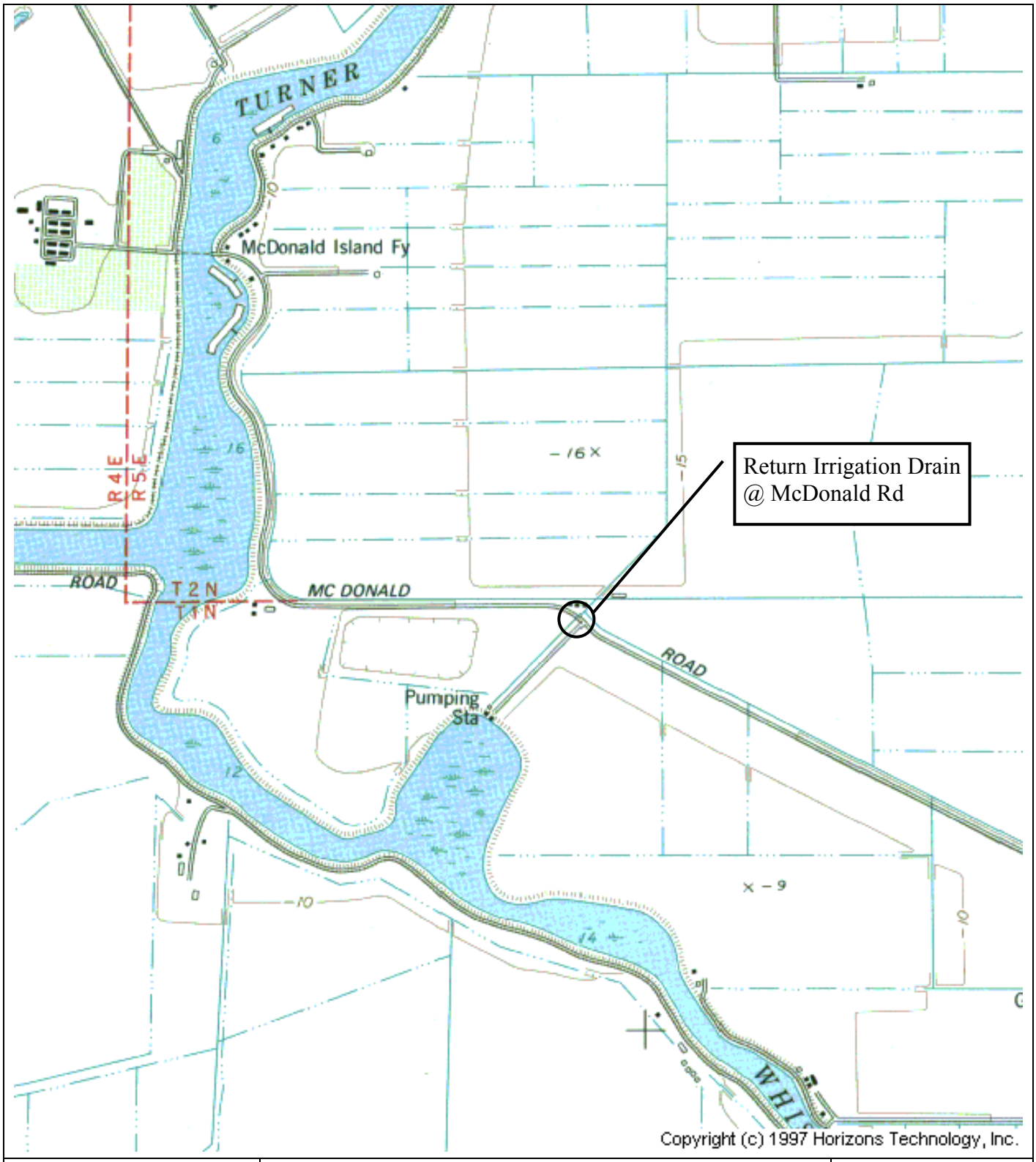
Drawing Reference: U.S.G.S TOPOGRAPHIC MAP 7.5 MINUTE QUAD Photorevised 2003	**SITE LOCATION MAP** Knight's Landing Ridge Cut @ Rd 16 North **YOLO COUNTY** 38.74894 N 121.69498 W	N W E S approx. scale



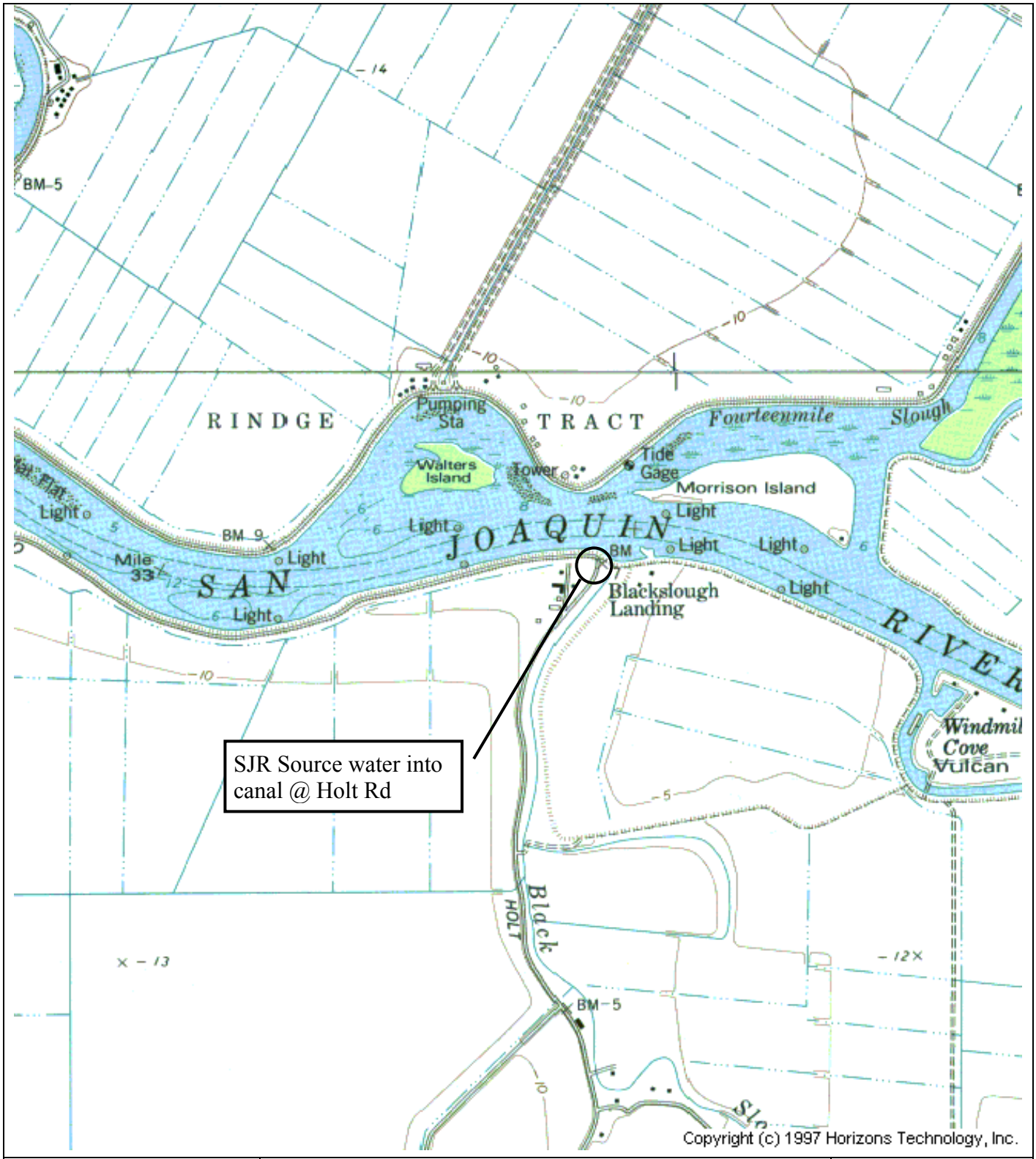
Drawing Reference: U.S.G.S TOPOGRAPHIC MAP 7.5 MINUTE QUAD Photorevised 2003	**SITE LOCATION MAP** Beaver Slough @ Blossom Rd **SAN JOAQUIN COUNTY** 38.20421 N 121.44706 W	N W E S approx. scale



Drawing Reference: U.S.G.S TOPOGRAPHIC MAP 7.5 MINUTE QUAD Photorevised 2003	**SITE LOCATION MAP** Unamed Slough @ Woodsbro Rd **SAN JOAQUIN COUNTY** 37.92680 N 121.36697 W	N W E S approx. scale



Drawing Reference: U.S.G.S TOPOGRAPHIC MAP 7.5 MINUTE QUAD Photorevised 2003	**SITE LOCATION MAP** Return Irrigation Drain @ McDonald Rd **SAN JOAQUIN COUNTY** 37.96983 N 121.46227 W	N W E S approx. scale



Drawing Reference:

U.S.G.S TOPOGRAPHIC MAP

7.5 MINUTE QUAD

Photorevised 2003

SITE LOCATION MAP

SJR Source Water into Canal @ Holt Rd

SAN JOAQUIN COUNTY

37.99402 N 121.42045 W

approx. scale

APPENDIX C

Chain of Custody

Aquatic Toxicology Laboratory
University of California, Davis
Dept. of Vet. Med: Anatomy, Physiology & Cell Biology
Davis, CA 95616
(530) 752-0772 fax: (530) 752-0585

Chain of Custody

Program	Project Name	Sample Type (Composite/Grab)	Preservation (HNO3, Ice,,etc.)	Analysis					
Sampler (signature)	Print Name								

Sample Information				Container			Matrix			Sample Type (Composite/Grab)	Preservation (HNO3, Ice,,etc.)	Field EC	Field pH	Field Temperature			Sample Temperature Receipt
SAMPLE ID	**SAMPLE LOCATION**	Date	Time	Type	No.	Size	Water	Waste Water	Soil/ Sludge								

Special Instruction/Suspected Constituents:

Relinquished by (signature/company)	Date/Time	Received By (signature/company)	Relinquished by (signature/company)	Date/Time	Received By (signature/company)

APPENDIX D

Summaries of Toxicity Test Parameters and UCD ATL Standard Operating Procedure References

Table 1. Summary of the 96-hour *Ceriodaphnia dubia* survival test.

1. Protocol	US EPA (1994)
2. Species	Ceriodaphnia dubia
3. Age	Less than 24 hours old and all born within a 20 hour window
4. Test type	Static renewal
5. Test duration	96 hour
6. Endpoint	Mortality
7. Temperature	25 ± 2°C
8. Photoperiod	16 hours light and 8 hours dark
9. Test chamber size	20 ml scintillation vials
10. Test solution volume	18 ml
11. Renewal of test solution	Daily, approximately 100% renewal
12. Number of neonates/test chamber	5
13. Number of replicates/sample	4
14. Feeding	YCT and *Selenastrum*, See SOP 9-3 and 9-5
15. Aeration	Aeration is required only if DO exceeds 102% saturation at 25 ± 2°C, or if the sample DO is below 4 mg/L.

16. Water chemistry	DO, temperature, pH, EC, alkalinity, hardness, and ammonia are measured in ambient samples. Temperature, pH, and DO are measured in test samples at 24-hour water renewals.
17. Culturing procedures	See SOP #2-4
18. Sample filtration	53 μm plankton net
19. Light quality	Fluorescent with a light diffuser panel
20. Light intensity	50-100 ft-c

Table 2. Summary of the 96-hour larval fathead minnow (*Pimephales promelas*) survival test.

1. Protocol	US EPA (1994)
2. Species	*Pimephales promelas* larvae
3. Age	Less than 48 hours old
4. Test type	Static renewal
5. Test duration	96 hours
6. Endpoints	Morality
7. Temperature	25 ± 2°C
8. Photoperiod	16 hours light and 8 hours dark
9. Test chamber size	600 ml beaker
10. Test solution (volume)	250 ml/replicate
11. Renewal of test solutions	Daily, 80% renewal of original sample
12. Number of larvae/test chamber	10
13. Number of replicates/sample	4
14. Feeding	*Artemia nauplii* see SOP #9-4
15. Aeration	Aeration is required only if DO exceeds 102% saturation at 25 ± 2°C, or if the sample DO is below 4 mg/L.
16. Water chemistry	DO, temperature, pH, EC, alkalinity, hardness, and ammonia are measured in ambient samples. Temperature, pH, and DO are measured in test samples at 24-hour water

	change outs.
17. Culturing procedures	Received as larvae (SOP #2-5)
18. Sample filtration	53µm plankton net
19. Light quality	Ambient laboratory illumination with light diffuser panel.
20. Light intensity	50-100 ft-c (ambient laboratory levels)
21. Cleaning	Siphon daily with turkey baster immediately before test solution renewal

Table 3. SOP References for procedures/equipment.

PROCEDURE/EQUIPMENT	SOP Number
25°C Water Baths	8-15
Alkalinity	6-6
Ammonia	6-3
Balance	8-2, 8-3
Ceriodaphnia Acute 96 hour toxicity testing, Toxicant Identification Evaluation (acute) for *Ceriodaphnia*	1-7
Ceriodaphnia culturing	3-1
Cleaning of Glassware	10-1
Corrective Actions	12-1
Coulter Counter	8-7
Dissolved Oxygen Meter	8-10, 8-11, 8-21
EC Meter	8-8, 8-16, 8-17
Field Equipment and Sampling	5-1, 5-2, 13-6
pH Meters	8-9, 8-13
Preparation of Food Algae	9-3
Preparation of YCT	9-5

PROCEDURE/EQUIPMENT	SOP Number
Preservation of samples for metals analysis	6-7
Protocol Amendment	12-3
SOP/QAPP Deviation	12-2
Thermometers	8-5, 8-6, 8-12
Total and Calcium Hardness	6-1, 6-2
Toxicant Identification Evaluation (acute) 96 hour for fathead minnow	1-8

APPENDIX E

Instrument Calibration and Preventative Maintenance

Laboratory instruments are calibrated, standardized and maintained according to procedures detailed in the SOP Manual. Section 8 of the SOP's, "Instrument Protocols", identifies step-by-step calibration and maintenance procedures. EC and pH meters are checked against known standards every five weeks for precision. Data generated from the quality assurance checks will be incorporated into a control chart. Prior to use, field instruments are calibrate and recorded in the field log book.

- **Mettler AE 100 Balance**: Used for the routine weighing of chemicals. Before operation, the balance is verified to be level. Adjustments are made to level properly if necessary. An internal calibration is performed any time the balance is unplugged or moved. Prior to use the balance is checked with reference weights. The balance is serviced and calibrated by a quality control service annually.

- **Max/Min Thermometers**: Used to detect the maximum and minimum fluctuations in temperature over a given time period in environmental chambers, refrigerators and water baths. Mercury thermometers are calibrated using a NIST certified thermometer annually.

- **Model ZM Coulter-Counter**: Used to determine algal density for *Ceriodaphnia* food by counting the number of cells, of a given size in a given volume of fluid. Though the Coulter-Counter is not calibrated a control count is performed on a solution with a known concentration of microspheres (counting beads). The Coulter-Counter is oiled every 5 weeks and the tubing is maintained with isotonic solution detergent.

- **YSI Model 33 Electrical Conductivity (EC) Meter**: Used to determine the electrical conductivity and/or salinity of a water sample. This meter has an internal calibration that is performed daily. The internal cell constant is calibrated every five weeks with a traceable conductivity calibration standard. At this time the probe is also checked and cleaned when there are traces of hard water deposits, oils and organic matter.

- **Beckman 12 pH/ISE Meter**: Used to measure the pH of a water sample. It is calibrated daily against two buffers (7.0 and 10.0). Every five weeks it is checked against a secondary precision pH buffer of 7.0 and 10.0. pH meter probes are checked weekly for algae buildup

and for appropriate fluid levels. pH buffers and KCl storage solutions are changed every five weeks.

- **YSI Dissolved Oxygen (DO) Meter 58**: Used to determine the concentration of dissolved oxygen in a water sample. The probe is zeroed and calibrated in saturated deionized water at test temperature daily. The probe membrane is replaced every five weeks and checked for bubbles and wrinkles.

- **HACH Model 2100A Turbidimeter**: Used to determine Norton Turbidity Units (NTUs) of an ambient sample. The meter is calibrated with NTU standards that are within the range for the water sample.

- **EM Science Aquaquant Ammonium kit**: Used to determine ammonia content of a sample. A standard and a blank are run with samples to ensure the reagents are reacting properly.